

**EVALUATION OF LEAF BASE EXTRACT OF *SORGHUM*  
*BICOLOR* (FAMILY: GRAMINEAE; POACEAE) FOR  
HAEMATOLOGICAL, IMMUNOMODULATORY AND  
OTHER PHARMACOLOGICAL ACTIVITIES**

**By**

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**APRIL, 2008**

## DECLARATION

I declare that the work in the dissertation entitled ‘Evaluation of leaf base extract of *Sorghum bicolor* (Family: Gramineae; Poaceae) for Haematological, Immunomodulatory and other Pharmacological Activities’ has been performed by me in the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, under the supervision of Prof. (Mrs) H.O. Kwanashie, Prof. A.A. Ahmad and Dr. (Mrs) L.E. Odama.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

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Signature

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Date

## CERTIFICATION

This dissertation entitled **‘EVALUATION OF LEAF BASE EXTRACT OF *SORGHUM BICOLOR* (FAMILY: GRAMINEAE; POACEAE) FOR HAEMATOLOGICAL, IMMUNOMODULATORY AND OTHER PHARMACOLOGICAL ACTIVITIES’** by Nwinyi, Florence Chimezie meets the regulations governing the award of the degree of Doctor of Philosophy in Pharmacology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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## ABSTRACT

*Sorghum bicolor* is an annual plant having its different parts widely used ethnomedicinally for different ailments, which includes blood stimulation and body defence. A number of clinical conditions like cancer, surgery, certain drugs, HIV/AIDS and various stressors affect different components of the immune system thereby allowing opportunistic pathogens to overwhelm the host leading to secondary infections and mortality in such individuals. This therefore shows a great need for investigation of plants with body defence potentials to increase the catalogue of agents that can modify the immune system for subsequent drug development and clinical uses. Also, many scientific reports have shown some drugs to be efficacious, cheap and available, yet toxic. This therefore shows the importance of evaluation of safety profile of a drug intended for short and long term uses.

The base of the leaves attached to the suckers of the plant were successively cold macerated with 70 % v/v methanol over 96 h period producing a yield of 23.6 % w/w extract. The blood stimulatory and body defence properties of the extract were evaluated on haematological indices (Hb, PCV, total red blood cell, total leucocyte count, differential leucocyte count, platelet, clotting time, bleeding time) and immunomodulatory models for specific and non-specific types of immunity. The evaluation of safety profile was carried out on pharmacological studies related to different body systems (CNS, PNS, GIT, CVS and reproductive systems). It was also evaluated on both acute and sub-acute toxicity models. The results were expressed as mean  $\pm$  SEM. One-way or two-way analysis of variance (ANOVA) was used to analyse



the results as appropriate followed by Student t-test and Least Significant Difference (LSD). P-values <0.05 were statistically significant.

The extract (100 – 400 mg/kg p.o.) did not produce significant effects on most of the haematological indices (Hb, PCV, total red blood cell, total leucocyte count, bleeding time and clotting time). The results did not corroborate the ethnomedicinal use of the plant leaves for stimulation of blood production. Immunomodulatory studies showed that phagocytosis and inflammation (usually associated with non-specific type of immunity) possibly contributed strongly to the ethnomedicinal use of the leaf base for body defence. The results on the other hand showed that both B- and T-lymphocytes (associated with specific type of immunity) may not have been sensitised/activated strongly by the extract. Pharmacological studies revealed centrally-mediated activity, analgesic effect, anti-motility and anti-diarrhoeal activities of the extract. The extract did not alter the intrinsic myogenic contractions of rat atria, portal vein and uterus while the rat vas deferens were minimally contracted.

The results therefore indicate that *S. bicolor* leaf base extract has a potential of being developed as a remedy for pain, central nervous system and gastrointestinal related problems. The acute and sub-acute toxicity evaluation also showed relative safety.

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## GLOSSARY OF ABBREVIATIONS

ASA	- Acetyl Salicylic Acid
BSA	- Bovine Serum Albumin
SRBC	-Sheep Red Blood Cell
PBS	- Phosphate Buffered Saline
GOT	- Glutamate Oxaloacetate Transaminase
GPT	- Glutamate Pyruvate Transaminase
ALP	- Alkaline Phosphatase
PCV	- Packed Cell Volume
Hb	- Haemoglobin
GLP	- Good Laboratory Practice
OECD	- Organisation for Economic Cooperation and Development
ANOVA	- Analysis of Variance
WBC	- White Blood Cell
DLC	- Differential Leucocyte Count
p.o	- Per os or Per oral
i. p	- intraperitoneal
DTH	- Delayed Type Hypersensitivity
NIPRD	- National Institute for Pharmaceutical Research and Development
SEM	- Standard Error of Mean
CNS	- Central Nervous System
GIT	- Gastro-intestinal Tract
PNS	- Peripheral Nervous System
CVS	-Cardiovascular System

**1.1 The Body Immune System****1.1.1 Immunity**

Immunity is the state of the living organism whereby it resists or is non-susceptible to infection (Zink, 1980). Reactions that take place between infecting micro-organism and the host are quite complex. The degree and speed with which the invaders may do harm depend entirely on the resistance or lack of resistance of the host. It is not possible to differentiate sharply between states of immunity and of susceptibility since these terms are relative.

The virulence of invading bacteria, viruses or fungi, and the level of resistance offered by body tissues and cells are of utmost importance in determining the outcome of an infection. With the development of an adequate level of immunity, the host adapts itself to the presence of foreign proteins, displays marked resistance to the invasive powers of infecting micro-organism, and is capable of protecting itself against the deleterious action of their toxic -products (Zink, 1980; Ivan, 1988).

It is however worth noting that immunological system is equipped not only to perform a defense function against infectious agents, but also to concern itself with the more diverse biologic functions of homeostasis which involves removal of worn-out (effete) “self” components so as to preserve uniformity of a given cell type. It is this function that concerns itself with normal degradative or catabolic functions of the body charged with the removal of damaged cellular elements, such as circulatory erythrocytes or

leucocytes. These may be damaged during the course of a normal life span or may arise as a consequence of injury (Bellanti, 1978).

The immunologic system also performs a surveillance function. It monitors and disposes abnormal cell types which constantly arise within the body. Most of these newly acquired configurations occur on cell surfaces. These mutants may occur spontaneously or may be induced by certain viruses and chemicals. Failure of this mechanism therefore has a causal role in the development of malignant disease (Bellanti, 1978).

However, despite the fact that in the classic usage, immunity referred to the relative resistance of the host to re-infection by a given microbe, and also immune system concerning itself with functions of homeostasis and surveillance, it is now evident that immune responses are not always beneficial, nor are they solely associated with resistance to infection. They can even confer unpleasant and harmful effects on the host (Bellanti, 1978). It has for instance been shown that when the cellular elements of defense are 'hyperactive', certain undesirable features such as 'allergy' or 'hypersensitivity' occur. Persistence of an antigen in the body despite the attempt by immunological system to eliminate it may lead to a tissue damaging immunological response. These responses are no longer beneficial to the host and are manifested as disease phenomena, 'the immunologically mediated diseases (IMD)'. This represents a maximal deleterious, self perpetuating attack of an aberrant immune responses in which the host sustains injury. Aberrations of homeostasis are exemplified by the auto-immune diseases, in which these mechanisms are unduly enhanced.



In order for the state of the body to be kept as optimally as possible, the immunological system confers two types of immunity which include non-specific and specific immunity.

#### 1.1.2 *Non-specific Immunity (Phagocytosis and Inflammation)*

This is the primary response to the first encounter of the host with a foreign substance. This usually involves mobilization of phagocytic cells/phagocytes into areas where a foreign substance has been introduced for the removal of the foreign substance by the process called phagocytosis. This may occur as an isolated event or as part of inflammatory response which involves a spectrum of cellular and systemic events that occur in which the host attempts to restore and maintain homeostasis following any one of a variety of tissue injuries (either by mechanical or chemical agents or by self destruction/auto-immune processes). This therefore shows that, although there is a tendency in clinical medicine to consider the inflammatory response as harmful reaction to the body, it is essentially a 'protective' and 'restorative' response in which the body attempts either to return to the pre-injury condition or to repair itself after inflicted injury (Ward, 1978).

Inflammatory response is accompanied by a number of systemic events that involve fever as well as a series of hematologic phenomena. The febrile response reflects enhanced metabolic activity following injury. One mechanism is the release of endogenous pyrogen from host leukocytes. An increased leukocyte count occurs during bacterial infections or tissue injury. Swelling, redness, heat, pain and altered function are usually associated with inflammation. The inflammatory response is dependent on both intact blood vessels and the circulatory cells and fluids within these channels.

Inflammation is heralded by dilatation of blood vessels and the outpouring of leukocyte and fluids. Grossly, the result is 'redness' (erythema) due to blood vessel dilatation, 'swelling' (oedema) due to escape of fluids into soft tissues and 'firmness' (induration) due to accumulation of fluids and cells. The result of these processes leads to a loss of the normal capacity of the blood vessels to retain fluids and cells within the vasculature; however, such changes do not necessarily reflect structural impairment of the vessel. Leukocytes may be responding to chemical attractants that are diffusing towards the vessel from an extravascular site. Moreover, the release of certain factors such as 'histamine' from tissue mast cells may subsequently render the vessels more permeable to plasma fluids (Ward, 1978; Insel, 1990).

In most cases, the acute inflammatory response reflects the effects of mediators acting on the blood vessel, rather than a non-specific injury to the vessel resulting in the selective release of fluids and cells. Vaso-permeability changes may appear early in the acute inflammatory response following mechanical trauma or thermal injury. Neutrophilic granulocytes appear within 30 to 60 minutes of injury. They first cluster along endothelial cells of vessels in the injured area. The leukocytes thereafter thread out of the vessel by squeezing through junctions between endothelial cells. The granulocytes appear extravascular within minutes and accumulate in the area of injury (Ward, 1978).

Once the neutrophils are out of the confines of vessels, they represent the first line of defense against invading micro-organisms. Their prime function is to ingest (phagocytose) and destroy potentially dangerous agents such as bacteria. If the acute inflammatory response progresses for four to five hours, mononuclear cells such as

lymphocytes and monocytes appear at the inflammatory site after leaving the vessels through mechanisms similar to that of the neutrophils. Monocytes augment the defense by adding their own phagocytic function to the area, while lymphocytes convey the immunologic capacity to respond to foreign agents by specific 'humoral' and 'cell-mediated' phenomena (Ward, 1978).

All the above descriptions have stressed the protective function of the inflammatory process. However, if the inflammatory response is 'aberrant', a serious consequence may occur. For instance, an outpouring of too much fluid from the vasculature into an area such as the brain may lead to a serious rise in intracranial pressure. The accumulation of fluid due to inflammation in the pleural and pericardial cavities may seriously compromise organ function. Also, the arrival of excessive numbers of neutrophils and the subsequent discharge of their enzymatic contents may result in serious structural damage. This occurs in cases of immunologic vasculitis or nephritis, in which dissolution of basement membrane occurs as a consequence of enzymatic hydrolysis, sometimes with catastrophic results (Henson, 1978).

It has been shown that many diseases confronting the clinician are due to an uncontrolled inflammatory response. The joint damage in rheumatoid arthritis, the functional and structural damage in glomerulonephritis, and the demyelinating diseases of the central nervous system are examples of excessive or uncontrolled inflammatory response. The treatment usually involves anti-inflammatory therapy since information about the causative agents of these entities is not well known (Waksal, 1978; Fauci, 1978).

### 1.1.3 *Specific Immunity*

The specific immune responses are concerned with the recognition and ultimate disposal of foreign agents in a highly discriminatory way. The immune mechanism involves the presence of an antigen (immunogen) which provokes the production of antibody molecules or sensitized lymphocytes depending on the nature of the stimulatory antigen. This results to 'humoral' and 'cellular' immunity respectively. The criteria for what constitutes an antigen are dependent not only upon the individual source or chemical configuration but the species or biologic relationship of the animal being stimulated (Karush, 1962).

*Antibody and Humoral Immunity:* Antibodies can be defined as specific serum proteins produced as a result of stimulation by an antigen. The production of antibodies is the function of the humoral or plasmacytoid division of the immune mechanism. Usually, antibodies are directed against antigens that gain access to the blood such as transfused blood cells, and some kinds of bacteria and viruses. They are capable of chemical union both *in vivo* and *in vitro* with a particular antigen responsible for their production. Antibody molecules are produced in special free circulating lymphocytes upon stimulation by specific antigenic structure called the 'determinant'. These antibody producing cells referred to as 'immunocytes' are derived from bone marrow stem cells. The cells produced by bone marrow that are destined for antibody production are called the 'B' cells which after differentiation and maturation, form plasma cells which secrete antibodies (Stewart, 1980).

It is worth noting that in some individuals, an infection may be so mild that the disease never reaches the clinical stage (sub-clinical infection). Antibodies however, may be

produced and in sufficient amount so that the individual will become immune to possible future infection by the same species of micro-organisms (Zink, 1980).

In routine immunological studies, reports have been made of the presence of protective antibodies to a variety of infections in individuals who have never had a clinical recognized case of the disease in question. This is the case particularly with many of the viral diseases including HIV/AIDS. The degree of immunity possessed is the determining factor in successful resistance against future exposures. Other factors, relating to the general level of health contribute, along with specific immune substances, to the resistance offered against invading pathogenic organisms (Zink, 1980).

*Lymphocytes and Cellular Immunity:* This involves the system responsible for rejection of organ transplants or skin graft as well as the defense mechanisms against many types of parasites and endogenous neoplastic (tumor) growths. It also relies on antigen stimulation for its activation. Small lymphocytes are the cells responsible for cellular immunity and also originate from the bone marrow stem cells and are called 'T-cells'. They have been incriminated in 'Delayed Type Hypersensitivity Reactions' as in Allergic Contact Dermatitis (Stewart, 1980; Ivan, 1988).

Following initial contact with a chemical compound which is capable of producing cell-mediated hypersensitivity, a process of sensitization occurs in some individuals and in the course of a minimum of six days, lymphocytes located in lymph glands become sensitized and are released into the blood stream. Following subsequent exposure to the specific sensitizer, circulating lymphocytes accumulate at the site and dermatitis becomes manifest one to two days later at the skin site of re-exposure.

Such delay in the appearance of dermatitis at the site of the re-exposure is indicated by an alternative term for the reaction, namely 'Delayed Hypersensitivity' (Mitchel, 1975). Delayed hypersensitivity may be detected by means of a 'patch test', a chemical compound is applied to the skin in a non-irritant dose, and skin site is examined 48 h later. A positive patch test reaction indicates a state of delayed hypersensitivity (Mitchel, 1975; Shough, 1980).

## **1.2 Drugs and Immune System**

Immuno-pharmacology can be defined as the study of the effect of drugs on the immune system of living organisms. The immune system on the other hand, is complex in nature, consisting of multi-organ and involves cells in its activity. It is one of the most sensitive systems of the body and works throughout the body through an intricate regulation of cellular and humoral components. Its protective task puts it in a vital position between a healthy and diseased state of the host.

Modification of immune function (with drugs) has been of great use in therapeutics. This is probably why the concept of modulation of immune responses to alleviate diseases long existed in ancient Indian system of medicine (Ayurveda) and Greco-Arab system of medicine (Unani-Tib; Said, 1969). Herbal drugs are known to have immunomodulatory properties. These immunomodulatory agents are of plant origin and act by stimulating both non-specific and specific immunity (Wagner, 1983; Atal *et al.*, 1986). It is now being recognized that immunomodulatory therapy could provide an alternative to conventional chemotherapy to variety of disease conditions (Saraf and Bhide, 1983).

Different pharmacological agents have been known to cause either immune stimulation or suppression both of which could be useful depending on the therapeutic requirement. Some are also known to cause immuno-tolerance. The importance of having the knowledge of immune related drugs cannot be over-emphasized. This possibly explains why in recent years, the field of immuno-modulation attracted attention of scientists all over the world. There is growing awareness regarding the need to modulate the host immune system to achieve the desirable effects of preventing an infection rather than treating it at an advanced stage (Chatterjee, 1996). This also led to the introduction of the concept of 'pro-host therapy' (Drews, 1982; Hadden, 1983) which aims to boost host immune functions to prevent infections.

However, it is worth noting that most of the side effects seen in drug therapy have been related to the pharmacological activity of the drug while about 15 % of all side effects of drugs are thought to be immune mediated (Schlumberger *et al*, 1993).

According to Stewart (1980), an immunosuppressive drug is one that can attenuate the expression of at least one type of immune response. Some classical cytotoxic immunosuppressants include methotrexate, azathioprine and cyclophosphamide, all of which act through different mechanisms aimed at inhibiting nucleic acid synthesis thereby thwarting the stimuli for proliferation of the lymphocytes involved in immune response. This also explains why many of the immunosuppressive drugs now employed were first used in cancer chemotherapy because of their toxicity to rapidly dividing cells (Ivan, 1988; Gilman *et al*, 1990). Great care is however needed in the use of immunosuppressants because patients on immunosuppressive agents are usually immuno-compromised and they tend to be susceptible to infections (Stewart, 1980;

Ivan, 1988). According to Damre *et al*, (2003), only a limited amount of immunosuppressive products of plant origin have been reported.

Immuno-suppression will be desired in conditions as allotransplantation or allografting, which is the transplanting or grafting of an organ or tissue from one person to another who does not share the same transplantation antigens. The most common allografting procedure is probably blood transfusion where unfortunate consequences of mismatching occur. Other procedures may involve solid grafts such as skin (Ivan, 1988). Other conditions where immuno-suppression will be desired include auto-immune diseases which are usually associated with the development of an immune response to normal body or self tissues as in rheumatoid arthritis, nephrosis, thyroiditis, early stages of insulin dependent diabetes mellitus, etc (Hanschmacher, 1990).

Immuno-stimulants on the other hand describe drugs capable of increasing the resistance of an organism against stressors of variable origin. They achieve this enhancement primarily by non-specific mechanisms of action. Immuno-stimulants generally stimulate in a non-antigen dependent manner, the function and efficiency of the non-specific immune system in order to counteract microbial infections or immunosuppressive states (Wagner, 1996). With respect to the mechanisms of action, immuno-stimulants influence primarily the humoral and cellular immune system. It has been shown that immuno-stimulants are effective prophylactically as well as therapeutically and must be applied at relatively low doses to achieve optimal effects. They are therefore suitable for regulative or modulating medication, i.e. for restoring body homeostasis (Wagner, 1996).



Immuno-stimulation has formed one of the basis for the development of prophylactic and in some cases therapeutic vaccines for conditions such as cancer (Drew, 1998), chicken pox, measles and tetanus. According to Drew (1998), cancer vaccination is therapeutic, involving attempts to activate immune responses against antigens in the tumor to which the immune system has already been exposed. It is also on this basis that subunit vaccines are commercially produced in genetically engineered bacteria, yeast, plants or mammalian cells with the aim of producing purified proteins that upon oral or parenteral administration deliver one (or more) immunogenic protein(s) in a manner that triggers an immune response (Sala *et al*, 2003).

A number of clinical conditions, like cancer (Santos *et al*, 1985), surgery (Grzelak *et al*, 1984) or administration of certain drugs (Mayer and DeTorres, 1985) as well as various stressors are known to affect different components of the immune system. The suppression of the host immune functions allows opportunistic pathogens to overwhelm the host leading to secondary infections and mortality in such individuals (Bruke, 1978). Immuno-stimulation has been found to be of value in all these conditions.

Immuno-stimulation is also useful in body defense against infections in individuals with defective immune responses such as premature infants, children with primary immunodeficiency or protein malnutrition or patients on steroid treatment. Acquired Immunodeficiency Syndrome (AIDS) which is a fatal disease condition caused by a Human Immunodeficiency Virus (HIV) is also another of such immuno-defective conditions (Ivan, 1988). Hersh and Freireich (1968) having reported that immuno-suppression is a major drawback in radiotherapy and chemotherapy in cancer patients suggested that

drugs that can stimulate immunity will be of great help in improving cancer treatment strategies.

According to Wagner (1985), the presence of immuno-stimulant compounds in higher plants has been extensively reviewed. Damre *et al*, (2003) suggested that, such products, if well tolerated by the patient may be developed into alternative co-adjuvants in the treatment of disorders caused by an exaggerated or unwanted immune response, such as in auto-immune diseases, allergies, glomerulonephritis, chronic hepatitis, etc.

### **1.3 Plants and Medicine**

Plants constitute one of the greatest resources of nature. Throughout the ages, plants have provided man with substances which are essential for living. They include food, medicine and raw materials for the manufacture of clothing, shelter, etc. Naturally occurring substances of plant, animal and mineral origin have provided a continually source of medicine since the earliest times of human existence, but it is the plant kingdom, in particular which has proved to be of most use for treating all human ailments (Philipson and Anderson, 1989).

Today, plants are still playing a significant role in the healthcare of a large proportion of the population in developing countries like Nigeria. Plants synthesize a large variety of chemical substances. These substances include in addition to the basic metabolites, phenolic compounds, terpenes, steroids, alkaloids, glycosides and a host of other chemical substances referred to as secondary metabolites. Many of these compounds have prominent effect and some possess important therapeutic properties, which can be and have been utilized in the treatment and cure of human and other animal diseases.

Thus, the plant kingdom provides a tremendous reservoir of various chemical substances with potential therapeutic properties (Abdul, 1986).

Experimentation with plants and the passage of knowledge from one generation to the next have over time succeeded in distinguishing those plants which have beneficial effects from those plants which are toxic or merely non-effective. There is a vast knowledge about the plants to use for foods, implements, medicines and narcotics and the methods of plant preparation (Webb, 1973). Ethnobotany has been shown to be a useful guide to the selection of plants containing active compounds (Vlietinck and Vanden Berghe, 1991).

Medicinal plants are believed to be important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990). Medicinal plants represent a great deal of untapped reservoir of drugs and the structural diversity of their component molecule makes them a valuable source of novel lead compounds against newly discovered therapeutic targets (Farnsworth, 1989; Harvey, 1999). During the 1800s, the active principles of a number of plant drugs were isolated (Phillipson and Anderson, 1989). It has also been estimated that about 50 % of all therapeutic agents (e.g. anti-diabetic, anti-biotic, hormones and alkaloids) are derived directly or indirectly from natural sources (Iwu, 1996). It has however, been shown that modern drugs come from less than 15 % of plants, which are known to have been investigated pharmacologically out of an estimated 250,000 to 500,000 species of higher plants (Farnsworth and Bingel, 1977). In 1980, the total world market value of plant-based medicines was estimated at 8 billion dollars (Hussain, 1992).

In Africa, herbal medicine is recognized as an important component of the healthcare system especially among the rural dwellers who constitute about 70 % of the population. Furthermore, in both rural and urban communities, many people depend partly on herbal remedies for their primary healthcare needs (Wambebe, 1998). In the developed countries, the popularity of crude herbal products is on the increase. In these technologically advanced societies, consumers' preference is shifting from purely synthetic to natural based drugs and this is dictating the basis for the global resurgence in the utilization of such products (Wambebe, 1998). Both consumer surveys and industry reports indicated that the use of complementary/alternative medicine and especially the consumption of botanicals have been increasing rapidly world-wide, including the United States (Eisenberg *et al*, 1993, 1998; Goldbeck-wood *et al*, 1996; Scimone and Scimone, 1998). In many countries, especially in Asia, herbal medicine has become an integral part of the healthcare delivery system on the same basis as orthodox medicine. However, in most countries in Africa, this is not yet the case (Wambebe, 1998).

In May 1987, the fourth World Health Assembly urged member States to initiate a comprehensive programme for the identification, evaluation, preparation, cultivation and conservation of medicinal plants used in traditional medicine and ensure good quality of drugs derived from medicinal plants by using modern techniques and Good Manufacturing Practices (GMPs).

Subsequently, in 1989, the World Health Assembly (WHA) adopted resolution WHA 42:43 in support of national traditional medicine programme and drew attention to herbal medicine as being of great importance to the health of individuals and

communities. The World Health Organization (WHO), in recognition of the immense value of herbal medicine to Primary Healthcare, advocated for the proper identification, sensible exploitation, scientific development and appropriate utilization of herbal medicines which provide safe and effective remedies. The stated aim of the World Health Organization to provide “Health for all by the year 2000” helped to focus attention on the systems of traditional medicine which are used extensively in all of the countries in the developing world (Bannerman *et al*, 1983). Since traditional medicines were incorporated into WHO’s programme in 1976, there has been more cooperation between practitioners of orthodox and traditional medicines.

It is obvious from these realities that due to the inherent value of herbal medicine and the immense socio-economic demands for adequate pharmaceutical supplies in the rural areas, transportation difficulties, the needed expertise for the rational use of drugs, the availability and cost of these products and according to Stepp and Moerman (2001), the belief that plants could hold cure to many chronic diseases such as cancer, AIDS, etc, herbal medicine remains the most valuable way to bridge the gap in medical practice.

It must however, be emphasized that important as it is, to aim at bridging the gap between the availability and the need for orthodox medicine, what has a lasting influence on our people and others in the world is self-reliance vis-à-vis development of herbal medicines, which are safe and effective.

**1.4            *Sorghum bicolor* (Linn.) Moench Syn. *S. vulgare* (Linn.) Pers. (Family:  
Gramineae; Poaceae)**

Sorghum is the common and scientific name for numerous cultivated annual grasses of the genus *Sorghum*, family: Gramineae. The sorghum are coarse plants, 3 to 15 feet (1 – 5 meters) tall and with or without lateral branches – called suckers, if they are near the base. The plants somewhat resemble corn. They have linear leaves that roll up in dry weather, thus reducing water loss, and both leaves and stem have a white waxy bloom. There is an extensive, shallow root system. The stalks bear large, compact clusters of bisexual flowers. The upper spikelets of the cluster are stalked and sterile, while the lower non-stalked ones are fertile and usually strong bristled. The flowers are followed by numerous small, glossy seeds that are reddish brown, white, yellow or black according to variety. Young leaves of all sorghums, whether on young plants or the suckers of old plants, occasionally contain sufficient hydrocyanic acid to kill livestock. This probably explains why Hausas (from personal communication) refer to the plant as ‘Karan (stalk) dafi’ (poison). There is no danger from grain, hay or silage.

It is highly adaptable, it does best on moist fertile land but it is of greatest importance in poor, semi-arid, or depleted areas, where it out-yields all other crops. It is grown in all tropical and warm temperate countries – including Africa (especially Egypt and Sudan), India, China, Southern Europe and North and South America (Encyclopedia Americana, 1995). Ethnobotanical information acquired in Nigeria showed that the plant is usually planted in the month of April and eventually matures for harvest. The leaves are harvested in parts as the plant grows and matures. The plant reaches its maturity between December and January when it is cut down.

Sorghums are usually grouped into four chief types: grain sorghums, sweet sorghums (with sugary sap, used primarily for forage or syrup); grass sorghums (for pasturage and hay); and broom corn for making broom heads. *Sorghum bicolor* is of the grain sorghums (guinea corn) which is made of different varieties used as food, stock feed, forage, etc. Sorghum is one of the earliest known plants to have been cultivated and is one of the world's most important crops. It is used for food, stock, feed, forage, hay, silage, syrup and sugar and for making paper, industrial alcohol, whiskey, beer, starch, edible oil, brooms and other products. The nutritive value of grain sorghum is 90 % to 95 % that of corn, with a large protein content, equal carbohydrate and less starch. The part of the crop not used for feed may be used to make industrial alcohol, whiskey, edible oil and starch (Encyclopedia Americana, 1995).

In Nigeria, *S. bicolor* is known by different vernacular names. These were obtained by personal communication and include: Baba; Oka-baba; Oka pupa (Yoruba), Igwu nowa (Idoma, Benue State), Ayii ovivi (Ebira, Kogi State), Damunngeri; ja dawa; Karan-dafi (Hausa).

Ethnomedicinal reports show that decoction from *S. bicolor* seed is demulcent and diuretic (Grieve, 1984). The inflorescence is astringent and haemostatic (Chiej, 1984). In Southern Rhodesia, the root is used for malaria; the seed used for breast disease and diarrhea; the stem for tubercular swellings. In India, the plant is considered anthelmintic and insecticidal and in South Africa, in combination with *Erigeron canadense* L., it is used for eczema (Watt and Breyer-Brandwijk, 1962). Duke and Wain (1981) reported folkloric use of *S. bicolor* as anti-abortive, intoxicant and poison. They also reported sorghum as a folk remedy for cancer, epilepsy, flux and stomach ache. In China, where

the seeds are used to make alcohol, the seed husk is braised in brown sugar with a little water and applied to the chest of measles patients. The stomachic seeds are considered beneficial in fluxes (Perry, 1980). Curacao natives drink the leaf decoction for measles, grinding the seeds with those of the calabash tree (*crescentia*) for lung ailments Venezuelans toast and pulverize the seeds for diarrhoea. Brazilians decoct the seed for bronchitis, cough and other chest ailments, possibly using the ash for goitre. Arubans poultice hot oil packs of the seeds on the back of those suffering pulmonary congestion (Morton, 1981). A decoction of 50 g seed in a litre of water is boiled down to half a litre as a folk medication for kidney and urinary complaints (Grieve, 1931).



**Plate 1:** *Sorghum bicolor* in its natural habitat



In Nigeria, the leaves of the plant are used traditionally to affect the blood and body defence systems as well as fertility (Personal Communication; Ibrahim Muazzam, a plant Taxonomist with the National Institute for Pharmaceutical Research and Development [NIPRD], Abuja, Nigeria). According to Okokoh (1999), a herbal healer, the plant not only purifies the blood but also stimulates the production of blood cells (blood builder). He reported that a patient may not need any blood transfusion if the herb is cooked and taken as tea on a daily basis for at least one month. According to his report, the herb can build up the blood to a level that anaemic and sickle cell patients can live normally. The leaves are also boiled with small potash and the extract given two times daily to women that lost too much blood during delivery. The leaves are also boiled with sugar cane and a 200 ml cup given three times a day for one week to women with amenorrhoea. The grains and the leaves are used to make medicinal pap called 'salala' (Hausa) and taken three times daily for one week to enhance fertility (Personal Communications). The leaves if boiled with a handful of *Capsicum annum* fruits (short ones), a handful of *Ficus sur* leaves with small potash and taken orally three times daily enhances the defence system of the body (Personal Communication, Ibrahim Muazzam, a plant Taxonomist).

Skinny domestic animals are fed with both the grain and the leaves of the plant for one month. The leaves are also used for tannary and colouring items like mats and clothes. The young leaves are used for arrow poisoning. Hence, the name 'karan dafi' (poisoning stalk in Hausa; Personal Communication).

*S. bicolor* is one out of the four herbal components (*Piper guineenses* seeds, *Pterocarpus osun* stem, *Eugenia caryophyllum* fruit and *Sorghum bicolor* leaves) of

sickle cell drug (NIPRISAN<sup>®</sup>) developed by National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria (Wambebe *et al*, 2001). *S. bicolor* is also one out of the three plant components of Jubi Formular<sup>®</sup>, (*Parquetina nigrescens*, *Sorghum bicolor* and *Harungana madagascariensis*), a commercial herbal preparation (haematinic) manufactured by Health Forever Products Ltd., Lagos, Nigeria (Erah *et al*, 2003). According to the company's claim, the product has the ability to enhance packed cell volume (PCV) and haemoglobin (Hb) and therefore recommended it for the support of treatment of moderate to severe anaemia as in sickle cell anaemia, cancer and HIV/AIDS. It is also recommended as nutritional supplement in stress, exhaustion and convalescent situations. It is also helpful in diabetes, hypertension, arthritis and infertility. Presently, the manufacturer's claim on the PCV and Hb enhancing ability of the product has been authenticated scientifically by Erah *et al*, (2003).

Some of the compounds compiled to have been isolated from *S. bicolor* include P-hydroxybenzaldehyde (30 %) isolated from wax seedlings; sorghumol isolated along with fernenol, trematol and isoarborinol and its structure determined. In terms of biological activity, P-hydrobenzaldehyde reduced normal feeding of locusts by 90 % (Rastogi *et al*, 1993). Cultivators with high pigmented seeds are rich in condensed catechin tannin and other phenols (anthocyanins; Morton, 1981). Toxicologically, sorghum is reported to contain hydrocyanic acid and the alkaloid hordernine. Sometimes the plant accumulates toxic levels of nitrate (Morton, 1981). The danger with hydrocyanide (HCN) is slight when grain is nearly mature. Young plants and suckers are dangerous, particularly when suffering from drought. HCN is destroyed when fodders is ensiled or cured as hay.

## **1.5 Relationship Between Haematological and Immunological Processes**

It has been reported that blood essentially consists of plasma, a fluid medium in which erythrocytes (red blood cells), leukocytes (white blood cells) and thrombocytes (platelets) are suspended (Baker *et al*, 1985). Plasma is reported to be a complex solution of proteins, salts and numerous metabolic substances and acts as a transport medium carrying its constituents to specialized organs of the body. Many of these plasma proteins such as the blood-clotting factors, enzymes and antibodies (immunoglobulins) have specialized functions (Baker *et al*, 1985).

It has also been reported by Bellanti (1978) that the activation of the host's immunological system by any foreign stimulus leads to a spectrum of cellular and humoral events that comprise the non-specific and specific immune responses. According to Bellanti (1978), the non-specific immune responses consist of phagocytosis and inflammatory responses. A number of effector mechanisms involving several cell types, cell products and soluble serum factors play some roles. The cellular constituents include mononuclear phagocytes (e.g. monocytes of the circulating blood and macrophages found in body tissues), granulocytes (neutrophils, eosinophils, basophils), platelets and lymphocytes. Some of these cells such as basophils, platelets, neutrophils and others such as the mast cells, entero-chromaffins participate in immunological reactions through the release of chemical substances (mediators) that have a variety of biological activities such as increased vascular permeability, contraction of smooth muscle and enhancement of the inflammatory response. The origin of these cells are hematopoietic stem cells located within the bone marrow, fetal liver and yolk sac of the foetus (Bellanti, 1978).

All these therefore show that there is an appreciable relationship/interaction between hematopoietic/haematologic activities of the body and its immunologic processes. It may therefore mean that boosting the body's haematological level can boost the immune state of a host. Hence, the need to explore pharmacological agents (including medicinal plants) known to affect haematological parameters in the light of immunology. This prompted the investigation of *S. bicolor* for immunomodulatory activities having known that it is traditionally used to purify blood, stimulate the production of blood cells, is used in combination with other plant parts to build up body's defense and is a component of NIPRISAN<sup>®</sup>, a herbal drug developed for sickle cell as well as Jubi Formula<sup>®</sup>, herbal drug developed and recommended for moderate to severe anaemia, in sickle cell, cancer and HIV/AIDS.

## **1.6 Aims and Objectives of the Study**

Since it is known that the immune status of an individual plays an important role in the health of the individual, there is a great need for further investigation of plants to possibly increase the catalogue of agents that can modify immune responses, advantage of which can be taken in therapeutics.

Also, considering that what is important to humanity is development of medicines which are effective, readily available, affordable and non-toxic, there is the need for evaluation of pharmacological effects of *S. bicolor* on different body systems such as central nervous system, cardiovascular system, gastrointestinal system and reproductive system. There is also need for toxicological screening of the plant for safety evaluation.

### 1.6.1 *Aim of the Study*

The aim of the study was to determine the haematological, immunomodulatory and other pharmacological activities, if any, as well as the safety values of *S. bicolor* in traditional medicine; and for possible drug development.

### 1.6.2 *Objectives of the Study*

The objectives of the study were to carry out scientific investigations to:

- (i) Ascertain the effect of *S. bicolor* on various haematological indices such as total leucocyte count (TLC), differential leucocyte count (DLC), total red blood cells (TRBC), platelet count, packed cell volume (PCV), haemoglobin (Hb) and clotting/bleeding time.
- (ii) Determine the immunomodulatory activities of *S. bicolor* through activated charcoal clearance assay for macrophage phagocytosis and anti-inflammatory test which are models for non-specific immune responses as well as delayed type hypersensitivity reaction, humoral response in normal and immunodeficiency states, estimation of T-lymphocytes by 'E' rosettes which are models for specific immune responses.
- (iii) Screen the plant, *S. bicolor* for some other pharmacological activities related to central nervous system (CNS), cardiovascular system (CVS), gastrointestinal (GI) system and reproductive system.
- (iv) Determine the toxicity profile of *S. bicolor* through acute and sub-acute studies.

**Plate 2:** Stacked mature dry leaves of *Sorghum bicolor* showing part used for the study

**2.1 Prospects of Plant Constituents as Immunomodulators**

Man has over a long time used herbs or plant products as medicine to promote health and to maintain body's resistance against infection by potentiating immunity, re-establishing body equilibrium and conditioning of the body tissues (Savnur, 1950; Bhagwandash, 1978).

A good number of these herbal remedies, e.g. *Echinacea purpurea*, *E. pallida*, *Viscum album* and *Baptisia tinctoria*, have stood the test of time. However, there is not yet enough scientific data regarding the identity and effectiveness of most of these herbs (Gupta, 1994; Wagner, 1996). Moreso, most medicinal plants investigated and documented neither reach stage I of clinical trial nor gain confidence for clinical use by the practitioners of modern medicine (Gupta, 1994). However, as difficult as it may seem, further detailed investigations on medicinal plants are still going on and this has helped to reveal their involvement in enzymatic, endocrinal, immunomodulating functions. These have helped to widen their profile of activity and opened new vistas of therapeutic applications (Gupta, 1994).

Among plant constituents documented to modulate immune responses are saponins from *Albizzia lebbek* through synthesis of reaginic antibodies (Tripathi *et al*, 1979), alcoholic extract of *Tylophora indica* (asthaticus) which prevents egg albumin-induced anaphylaxis in guinea pigs (Harnath and Shaimia, 1975) and horse serum-induced broncho-constriction in sensitized rat lung (Gupta, 1975; Nanyampalli and Shet, 1979). Chewing of leaves of *T. indica* for 6 days gives protection to 71 % cases of bronchial

asthma on antigen challenge (Shrypuri and Agrawal, 1971). The plant saponins from *Clerodendron serratum* (Gupta and Tripathi, 1973a), *A. lebbeck* (Tripathi *et al*, 1978) as well as the alkaloidal fraction of *Solanum xanthocarpum* (Chitravanshi *et al*, 1990) and *T. indica* (Geetha *et al*, 1981) have been shown to protect sensitized mast cells from degranulation on antigen shock thus confirming the immunosuppressive and membrane stabilizing effects as seen with sodium chromoglycate. Extracts of *Syzygium aromaticum* flower bud (Kim *et al*, 1998), *Vitex rotundifolia* fruits (Shin *et al*, 2000), *Aquilaria agallocha* stem (Kim *et al*, 1997) and *Siegesbeckia glabrescence* whole plant (Kang *et al*, 1997) inhibited systemic anaphylaxis (allergic reaction) induced by compound 48/80, a systemic fatal anaphylaxis inducer. It also inhibited possible cutaneous anaphylaxis (local allergic reaction) induced by anti-DNP IgE as well as the histamine release from rat peritoneal mast cells, indicating that the extracts inhibit immediate hypersensitivity by inhibition of histamine release from mast cells. *Lantana camara* leaves produced a significant reduction in both the cellular and humoral immunity in sheep (Ganai and Jha, 1991). It also caused a significant reduction in absolute lymphocyte count (anti-lymphocytic activity), dry weight of cotton pellet-induced granuloma formation in rats (immunosuppressive activity), relative spleen weight (lymphoid organ). These showed anti-lymphocytic and immunosuppressive effects of the plant leaves (Garg *et al*, 1997). Berbamine, one of the important alkaloids in *Berberis julianea* or *Berberis poiretii* has been shown to possess significant leukogenic immunosuppressive effects (Liu *et al*, 1983; Li *et al*, 1989). Luo *et al*, (1998) reported that Berbamine could significantly inhibit the lymphoproliferative response to Con A and LPS, decrease the PFC numbers to T-dependent antigen (sheep red blood cells). It also decreased the ratio of CD<sub>4</sub>-positive cells to CD<sub>8</sub>-positive cells. According to Luo *et al*, (1998), berbamine showed a suppressive effect on mixed



lymphocyte reaction (MLR), delayed type hypersensitivity reaction (DTH) response and retarded the rejection of skin grafts in mice. These indicate its potential as clinical transplantation agent (Luo *et al*, 1998).

Flavonoidal fraction of *Tephrosia purpurea* significantly inhibited sheep red blood cells-induced delayed type hypersensitivity reactions and produced a significant, dose-related decrease in sheep erythrocyte-specific haemagglutination antibody titre. Both showed the ability of the flavonoidal fraction to modulate both the cell-mediated and the humoral components of the immune system (Damre *et al*, 2003). *Withania somnifera* extract significantly reduced leucopenia induced by cyclophosphamide treatment. It significantly increased the bone marrow cellularity compared to cyclophosphamide alone treated groups suggesting that the extract may be causing stem cell proliferation (Davis and Kuttan, 1998). Ethanol extract and purified diterpene andrographolides of *Andrographis paniculata* induced significant stimulation of antibody and delayed type hypersensitivity (DTH) response to sheep red blood cells. The plant preparations also stimulated non-specific immune response of the animals measured in terms of macrophage migration index (MMI) phagocytosis of C-leucine labeled *Escherichia coli* and proliferation of splenic lymphocytes. These suggest that different components of the extract contribute to immunostimulation (Puri *et al*, 1993). *T. indica* (Gupta and Tripathi, 1973b) as well as saponins of *A. lebbek* (Tripathi *et al*, 1977) and *Gardenia latifolia* have also been found to potentiate beta-adrenergic activity. This effect brings about broncho-dilatation and is considered to be helpful for relieving bronchospasm in asthmatic patients.

*T. bellerica* and *Ocimum sanctum* have been shown to give protection against histamine as well as pollen (*Acacia arabica*) induced bronchospasm in guinea pigs and the latter has also inhibited antigen-induced histamine release from sensitized mast cells and gave relief in allergic bronchial asthma (Palit *et al*, 1983). The anti-allergic action of *O. sanctum* has been found to be associated with significant production of IgE antibodies (Sen, 1993).

Among the several plants also investigated for anti-asthmatic effects are saponins isolated from *Clerodendron serratum* (Gupta *et al*, 1968), *Gardenia turgida* (Gupta, 1971a), *A. lebbek* (Tripathi *et al*, 1977) and *Solanum xanthocarpum* (Gupta, 1971b). They accorded protection to sensitized guinea pig against histamine as well as antigen (egg albumin micro-aerosols). The protective effect of *C. serratum* saponin was found to be associated with the augmentation of anti-allergic activity in the tissues as the lung extracts from the treated animal inhibited histamine and SRS-A responses on guinea pig ileum to a greater extent and for longer period as compared to the extracts from the untreated control animals (Gupta *et al*, 1968). Some of these plant medicines therefore seem to be promising sources for development of anti-asthmatic and anti-allergic drugs.

A good number of plants also have anti-inflammatory activity. Such plants include *Tinospora cordifolia* – bitter principle (Gupta and Chrivastava, 1966), *Balsamodendron mukul* – olioresin, *Curcuma longa* – volatile oil (Chandra and Gupta, 1972) and *Sambucus ebulus* – rhizome extract (Ahmadiani *et al*, 1998).

Development of body resistance or immunity against infection has been reported after treatment with *Abutilon indicum* and *Sida cardifolia* as evidenced by enhanced

production of anti-*Salmonella typhi* 'O' antibody and protection against tissue damage (Dixit *et al*, 1978). Thus, some of these plant products seem to activate body defense mechanism to fight against the disease. *Helleborous purpurascens* (Ranunculaceae) extract was reported to have increased leukocytes, neutrophils and phagocytosis in animals transcutaneously implanted with it (Bogdan *et al*, 1990). Septilin is shown to increase the total count of leukocytes and percentage of polymorphs in the peripheral blood and is extensively used in the treatment of several acute/chronic infections. It was also shown to protect mice from cyclophosphamide-induced myelosuppression and subsequent leucopenia (Praveen Kumar *et al*, 1997).

There are some plant-derived immunostimulatory drugs available on the drug market and these include extracts of *Echinacea* species (*E. purpurea*, *E. pallida*) used therapeutically and prophylactically against chronic and recurrent infections of respiratory organs and urinogenital organs, retarded wound healing and infected wounds, malignant diseases (in combination with chemotherapy or irradiation, (Bauer and Wagner, 1991). The treatment of leucopenia cancer patients undergoing radiotherapy with a phytopreparation containing *Echinacea* extract in a combination with *Baptisia* species extract resulted in a significant increase of the number of leukocytes (Bendel *et al*, 1989).

Plants have also been sources for development of anti-cancer compounds or anti-viral interferons (Dhawan, 1987). The interferon stimulator (SNMC) derived from *Glycerrhiza glabra* has been reported to give protection to patients of sub-acute hepatic failure known to be fatal in majority of cases (Acharya *et al*, 1993). Successful treatment of sub-acute hepatic failure with interferon stimulators bring into focus the

trial for many other indigenous drugs for the treatment of viral hepatitis and many other similar diseases including cancers.

The possibility of development of a herbal drug for treatment of auto-immune diseases like multiple sclerosis and the most dreaded disease, AIDS cannot be ruled out.

In addition to the inherent properties of some plants that have made them to modulate immune system, there have also been advances in recombinant DNA technology whereby a variety of foreign genes encoding therapeutic proteins are transferred into plant species such as potato and tobacco (Arakawa and Langridge, 1998). Plant biotechnology techniques have been used to create plants which contain a gene derived from a human pathogen, the resultant plant tissues will accumulate an antigenic protein encoded by the foreign DNA (Mason *et al*, 1992; Thanavala, 1995; Haq *et al*, 1995, Mason, 1996; Arakawa and Langridge, 1998).

In pre-clinical trials, it was found that antigenic proteins produced in transgenic plants retained immunogenic properties when purified; if injected into mice, the antigen caused production of protein-specific antibodies (Thanavala, 1995). In some experiments, where the plant tissues were simply fed to mice, a mucosal immune response occurred (Haq *et al*, 1995; Mason, 1996; Arakawa and Langridge, 1998).

Plants have been selected as bioreactors because their eukaryotic nature often permits appropriate host-translational modification of newly synthesized foreign proteins, which thus retain their biologic activity (Arakawa and Langridge, 1998). Also, a number of studies have proven that plants are efficient large-scale cost effective bioreactors for

production and delivery of bacterial and viral antigens, which upon oral or parental administration, induce significant immune responses in the recipient. Potato and tobacco plants have been shown to synthesize and assemble Norwalk virus capsid protein (Mason, 1996), hepatitis B virus surface antigen (Mason *et al*, 1992; Thanavala, 1995) and bacterial enterotoxin such as the pentameric B subunits from enterotoxigenic *Escherichia coli* (LT-B) and *Vibrio cholerae* (CT-B). Transgenic potatoes producing CT-B and LT-B evoke both systemic and intestinal immune responses in mice that eat the raw plant tissue and protect the animals from toxin challenge (Haq *et al*, 1995; Arakawa *et al*, 1997; Arakawa and Langridge, 1998).

Most importantly, plant-based production of biologically active recombinant proteins such as pathogen-specific antibodies (Ma, 1995; Ma *et al*, 1998) and vaccine antigens (Haq *et al*, 1995; Tacket *et al*, 1998; Arakawa and Langridge, 1998) for preventive immunotherapy, enables convenient oral delivery through consumption of edible plant tissues. According to Tacket *et al*, (1998), oral vaccines when compared to vaccine delivery by injection offer the hope of more convenient immunization strategies. Oral vaccines act by stimulating the immune system at effector sites (lymphoid tissue) located in the gut and use of edible plants has made this possible.

**3.1 Plant Collection and Identification**

The dry mature leaves of *Sorghum bicolor* (Family: Gramineae) were collected from Maganawa town in Sokoto State, Nigeria from November, 2004 to January, 2005. The plant was authenticated by a plant taxonomist, Mr. Ibrahim Muazzam of Herbarium Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The specimen was deposited in NIPRD Herbarium with voucher specimen number 3815.

**3.2 Preparation of the Leaf Extract**

The dark red portions of the leaves attached to the suckers of the plants were cut out from the entire leaves (the portion of the leaves especially claimed to be used ethno-medicinally). They were then pulverized in a mortar. Two hundred grammes (200 g) of the pulverized sample was cold macerated successively in 5 litres of 70 % v/v methanol over 96 h period on a shaker (GFL D 3006 mgH, Germany) to ensure maximum extraction. The extract was then filtered using clean cotton wool. The filtrate was placed on water bath to allow evaporation of the solvents and consequent concentration of the extract for subsequent studies. A yield of 23.6 % w/w extract was obtained. The extract was kept in a cool dry room until used for experiments.

**3.2.1 *Reconstitution and Administration of the Extract***

Freshly prepared extract solutions were used during the study to ensure stability of the preparations. Distilled water was used to dissolve the extract to volumes and concentrations appropriate to achieve the desired dose levels in the studies. Drugs were

administered via intraperitoneal (i.p.) and oral (p.o.) routes depending on the study. Volumes of extract administered were dependent on the body weight of each experimental animal, the intended dose and the concentration of the extract solution. The frequency of drug administration was dependent on the nature of specific studies.

### **3.3 Phytochemical Screening of the Crude Extract**

The phytochemical analyses of the crude extract was done using the standard method of Trease and Evans (1983). The extract was screened for the presence of various chemical constituents such as alkaloids using general tests, saponins using the froth test, tannins using ferric chloride test, flavonoids using ferric chloride test, steroids using Salkowski's test, terpenes using Liebermann-Burchard test and anthraquinones using Borntrager's tests. The presence of carbohydrate was also tested using Molisch's test and reducing sugar using Fehling's test, phlobatannins and phenols were also tested for using general tests.

### **3.4 Chromatographic Separation of the Extract**

#### *3.4.1 Partitioning of the Extract*

The crude extract was further partitioned into non-polar, medium polar and very polar components. This was to determine the component(s) responsible for some of the observed pharmacological effects. The bioassay-guided models adopted were those with effects on intestinal propulsion, tail flick test and pentobarbitone-induced sleep.

The solvents used for the partitioning include hexane (non-polar solvent), ethylacetate (medium polar solvent) and aqueous/water (very polar solvent). Partitioning was achieved by dissolving the extract (10.15 g) first in distilled water and then mixing the

aqueous solution with different solvents in a separating funnel and then shaking the mixture gently and allowing it to stand for about 30 minutes to give two immiscible layers. These layers were separated and the process repeated until the upper partitioning solvent became clear. The first solvent used was hexane in the aqueous extract solution. Hexane is not miscible with water. It defats the extract and also extracts the non-polar components of the extract.

Ethylacetate was the next solvent used. It is also immiscible with water and extracts medium polar components of the extract.

The aqueous solvent extracted and contained the very polar components of the extract. All the components/portions (hexane, ethylacetate and aqueous portions) were concentrated to small volumes in a rota vapour and finally concentrated on water bath for subsequent bioassay-guided use.

The hexane portion of the crude extract was greenish, fatty/oily and very small with a yield of ~ 0.5 % w/w. This is a probable indication of presence of only very small quantities of non-polar components (including fat and chlorophyll) in the extract.

Ethylacetate portion appeared shinny, deep brownish black in colour, clumped up but not sticky. It had a yield of 95.9 % w/w (constituting the major component).

The aqueous component appeared deep brownish, clumped up but very lightly sticky. It gave a yield of 3.6 % w/w.



### 3.5 Drugs and Chemicals

Some of the drugs and chemicals used for carrying out the studies include;

5-HT (Sigma, USA)	Cyclophosphamide (Cadila Health Care, Germany)
Acetylcholine (Sigma, USA)	
Adrenalin hydrochloride injection (Sinochem mingbo Ltd, China)	Cyproheptadin $\alpha$ ketoglutarate (FAES FARMA, Spain)
Albumin kit (Randox Ltd, UK)	D (+) – Glucose monohydrate (dextrose, LNL, Nigeria)
Alkaline phosphate kit (Randox Ltd, UK)	Diazepam (Calmpose®; Ranbaxy, India)
Amphetamine (Sigma, USA)	
Apomorphine (Sigma, USA)	Direct and total bilirubin kits (Randox Ltd, UK)
Aspirin (Sigma, USA)	
Atropine (Sigma, USA)	Ficoll-Paque (Amersham Pharmacia Biotech AB; Uppsala Sweden)
Bovine serum albumin (KPL, USA)	
Calcium chloride (BDH, Poole, England)	Formaldehyde (M&B, England)
Chloroform (Sigma – Aldrich, Gillingham – Dorset)	Glacial acetic acid (Searle, Essex, England)
Cholesterol kit (Randox Ltd, UK)	Glutamate oxalacetate transaminase (GOT) kit (Randox Ltd, UK)
Carbachol (Sigma, USA)	Glutamate pyruvate transaminase (GPT) kit (Randox Ltd, UK)
Castor oil (Bell sons, England)	
Cimetidine (Smithkline & French, England)	Histamine (Sigma, USA)
Creatinine kit (Randox Ltd, UK)	Loperamide (Xepa-S Pattinson, Malaysia)

Magnesium chloride (BDH, Poole, England)	Propranalol HCL (Biomedicine, Belgium)
Magnesium sulphate (BDH, Poole, England),	Sodium Chloride, (BDH, Poole, England)
Methanol (Fluka Chemie, Switzerland)	Sodium dihydrogen orthophosphate (BDH, Poole, England)
Oxytocin injection (Rotex Medica, Germany)	Sodium hydrogen bicarbonate, (BDH, Poole, England)
Pentobarbitone sodium (Sigma, USA),	Stilboestrol injection (May and Baker Ltd, England)
Phenobarbitone (Vitabiotics, England)	Total protein kit (Randox Ltd, UK)
Phosphate Buffered saline, PH 7.4 (Sigma, USA)	Triglyceride kit (Randox Ltd, UK)
Potassium chloride (BDH, Poole, England)	Triton x – 100 (Lubley, England)
Potassium dihydrogen orthophosphate (BDH, Poole, England)	Urea kit (Randox Ltd, UK)
	Uric acid kit (Randox Ltd, UK)

### 3.6 Animals

Wistar rats (83.6 – 316.5 g) of both sexes, Balb/C and Swiss albino mice (15.0 – 34.7 g) of both sexes, rabbits (2.2 – 2.8 kg) of both sexes and guinea pigs (345.0 – 418.0 g) were used for the studies. They were obtained from the Animal Facility Centre, Department of Pharmacology and Toxicology, NIPRD, Abuja and National Veterinary Research Institute, Vom, Plateau State, Nigeria.

The experimental animals were separated for at least two weeks in the experimental room for acclimatization. They were housed in appropriately designed cages and

beddings e.g. wood shavings for mice and rats; dry grass for guinea pigs and rabbits. The animals were maintained under normal environmental temperature, approximately normal 12 h day and night illumination cycle. The animals were fed *ad libitum* with NIPRD formulated feed which was standard for each of the animal species except when starvation was needed in the study. Municipal water supply was the source of water given to the animals. The maintenance and experimental rooms were cleaned and disinfected regularly. Soiled saw dusts were replaced often. The feed and water containers as well as the animal cages were also washed regularly.

In the experimental grouping of the animals, their age/body weight and sex of the animals were taken into consideration to achieve approximately equal conditions among the groups.

The animals were identified using picric acid solution to mark unique numbers on individual animals. Small cards indicating the study number, group number, animal number and dose level were stuck to different cages for identification.

Sheep blood samples for the preparation of sheep red blood cell (SRBC) suspension used for some of the studies were also collected from Garki Abattoir, Abuja, Nigeria.

The 'principles of laboratory animal care' (NIH Publication # 85-23, 1985) were followed in this study.

### **3.7 Haematological Studies**

The method of Satish *et al* (1997) was adopted for the study. Rats (83.6 – 126.7 g body weight) of either sex were grouped into four (of five rats each). The first group received normal saline (10 ml/kg p.o.) and served as the control. The second, third and fourth groups received the extract (100, 200 and 400 mg/kg p.o.) once daily for 14 days. The doses were based on the acute toxicity studies carried out on the extract at the beginning of the study (details shown under toxicity report ahead). On the 15<sup>th</sup> day, animals were anaesthetized with chloroform, sacrificed and blood collected in EDTA anti-coagulant bottles.

The standard methods of Baker *et al* (1985) and Jain (1986) were then used to measure the haematological indices which include; haemoglobin (Hb), packed cell volume (PCV), total leucocyte count (total WBC), differential leucocyte count, platelet count, total red blood cells, bleeding time and clotting time.

### **3.8 Immunomodulatory Studies**

#### **3.8.1 *Delayed Type Hypersensitivity Reaction (DTH) – An in vivo model for cell-mediated immunity***

The method of Dan *et al* (1973) was adopted for the preparation of 5 % v/v SRBC suspension used for the study. About 5 – 6 ml of the sheep blood collected from Garki Abattoir, Abuja, was washed with 15 – 20 ml of normal saline by centrifuging at 2,500 g for 10 min. The supernatant was aspirated and discarded. This process was repeated until the supernatant became very clear and was discarded. The SRBCs were then packed at the same centrifuge speed and time. A 5 % v/v SRBC suspension was made using 1 volume packed SRBC to 19 volumes of normal saline (1:19).

The modified method of Chong *et al* (1998) was used for the DTH reaction studies. Twenty albino mice of either sex weighing 15.9 – 32.8 g were selected and used. The mice were divided into four groups (of five mice each). The first group received normal saline (20 ml/kg p.o.) to serve as the control. Groups two, three and four were given the extract (100, 200, 400 mg/kg p.o.) respectively for 14 days.

On the 7<sup>th</sup> day of treatment with the extract, all the mice were sensitized intraperitoneally with 0.1ml of 5 % v/v SRBC (i.e. day zero (D0) for immunisation). On the 14<sup>th</sup> day of the extract treatment, a challenging dose of 0.05 ml of 5 % v/v SRBC was injected into the right hind foot pad (i.e. day 7 (D7) for immunization). The degree of induration (thickness of the foot pad) was measured at 0 h before and 24 h, 48 h and 72 h after the challenging dose using a vernier caliper. The degree of induration (cm) in the treated group compared with those of control was used as the measure of DTH reaction.

### 3.8.2 *Studies on Humoral Response in Normal and Cyclophosphamide – induced Immunodeficiency*

This study was carried out using the modified method of Pallabi *et al* (1998). In brief, the method involved the preparation of 1 % and 10 % v/v suspension of SRBC respectively. About 5 – 6 ml of the blood collected from Garki Abattoir, Abuja was washed in 15 – 20 ml of normal saline for 10 min in a centrifuge at 2,500 g as described previously. The SRBCs were then packed at the same centrifuge speed and time. A reconstitution to 1 % and 10 % SRBC suspension was done using phosphate buffered saline (PBS, pH 7.4) in the ratio of 1:99 and 1:9 respectively.

Five groups (n=5) of BALB/C mice (of either sex weighing 15.7 – 31.1g) were used. The first group received normal saline (20 ml/kg p.o.) from day (-9) to day (+5). The second to fourth groups received the extract (100, 200, 400 mg/kg p.o.) respectively daily from day (-9) to day (+5). The fifth group received cyclophosphamide (100 mg/kg p.o.) on day (+2) to serve as a reference standard. On day 0, all the mice in every group were immunized (i.p.) with 0.1 ml 10 % suspension of SRBC in PBS. On day (+6), blood was collected from each mouse and serum prepared from it. Serial two fold dilution of serum were made in 25 µl of normal saline containing 0.1 % Bovine Serum Albumin (BSA) in microtitre plates. This was mixed with 25 µl of 1 % SRBC suspension in PBS (pH 7.4). This was allowed to stand at 37 °C until the control wells showed small buttons (negative pattern). The value of highest serum dilution carrying visible haemagglutination was taken as the antibody titre expressed in terms of number of wells.

### 3.8.3 *Activated Charcoal Clearance Assay for Macrophage Phagocytosis*

The modified method of Pallabi *et al* (1998) was adopted for this study. Wistar rats of either sex weighing 120.0 – 205.7 g were grouped into four (of five rats each). The first group was given distilled water (10 ml/kg p.o.) to serve as the control. Groups two to four received the extract (100, 200 and 400 mg/kg p.o.) for 14 days. On the 15<sup>th</sup> day, 0.3 ml of 0.5 % activated charcoal suspended in normal saline was injected intravenously through the tail vein of each of the mice. 25 µl blood sample was collected from the orbital plexus of each mouse just before the injection of 0.5 % activated charcoal above and at 5, 10, 15 and 30 min. after injection of the charcoal. The blood samples were each lysed in 3 ml of distilled water. The optical density was then measured with UV

visible recording spectrophotometer (Schimadzu UV – 160 A) at 650 nm using pre-injection blood sample as blank. The graph of absorbance was then plotted against time (min). The rate of charcoal clearance termed phagocytic index was calculated as the ratio of slope of regression line of treated groups to the slope of regression line of the control. Values:

< 1.0 = no effect

1.0 – 1.5 = slight stimulation of phagocytic rate (active)

> 1.5 = strong stimulation of phagocytic rate (very active)

#### 3.8.4 *Anti-inflammatory Studies*

The study was done according to the method of Winter *et al* (1962) as was modified by Akah and Nwambie (1994). The Wistar rats used for the investigation were deprived of water during the experiment to ensure uniform hydration and minimize variability in oedematous response (Winter *et al*, 1963). They were divided into five groups (n=5) of either sex and body weight between 172.3 – 316.5 g. The first group received normal saline (20 ml/kg i.p.) and served as negative control. Three doses of the extract (100, 200 and 400 mg/kg) were administered intraperitoneally to the second, third and fourth groups respectively while acetyl salicylic acid (ASA, 100 mg/kg i.p.) was given to the fifth group as a reference standard.

Inflammation was then induced 30 min later by injecting 0.1 ml of fresh egg albumin into the sub-planta surface of the right hind paw of each of the rats. The principle and technique of volume displacement were adopted for the measurement of paw volume (cm<sup>3</sup>) using LETICA Digital Plethysmometer (LE 7500) earlier calibrated with 0.15 % Triton x – 100. Zero readings were taken twice before injection of egg albumin (0 min)

and at 20 min intervals after the injection of egg albumin over a 2 h (120 min) period. The oedema at every interval was calculated in relation to the mean paw volume before the injection of the egg albumin. Activity for the treated groups was expressed as percent inhibition of inflammation in relation to the control group.

### 3.8.5 *Estimation of T-lymphocytes by 'E' Rosettes*

The principle of Kaplan and Clark (1974) was adopted for this study. Four groups of rats (n=5) weighing 131.0 - 240.0 g of both sexes were used for the investigation. The first group received normal saline (20 ml/kg p.o.) to serve as the control. The remaining three groups received graded doses of the extract (100, 200 and 400 mg/kg p.o.) respectively for 14 days. On the 15<sup>th</sup> day, blood was collected from the orbital plexuses of the rats into test tubes containing 0.5ml each of Alsever solution which according to Lennette and Schmidt (1979) constituted of; dextrose (20.50 g), sodium chloride (4.20 g), citric acid (0.55 g), sodium citrate (8.00 g), distilled water q.s. *ad* (1,000.00 ml) sterilised by autoclaving for 10 min at 10 Lb pressure.

1 ml of PBS (pH 7.5) was then added to each of the above tubes and gently mixed. 0.5 ml of Ficoll-paque (research grade) was put in new sets of glass centrifuge tubes. About 1 ml of the rat buffer-diluted blood above was aspirated with Pasteur pipette and carefully layered on top of the Ficoll-paque. Extreme care was taken not to mix the blood into the Ficoll-paque. These were then centrifuged at the speed of 1,500 rpm for 10 min. in a bench centrifuge. The centrifuge was allowed to coast to a stop to avoid disruption of the separated layers.



This separated the mixture into three (3) distinct layers made of the upper layer (constituted of the fluid in which rat blood was suspended), middle pale white band/layer (an opaque interface constituted of the mononuclear cells as the lymphocytes and monocytes suspended on top of the ficoll-paque) while the bottom layer is constituted of clumped red blood cells and other granulocytes (figure 1).

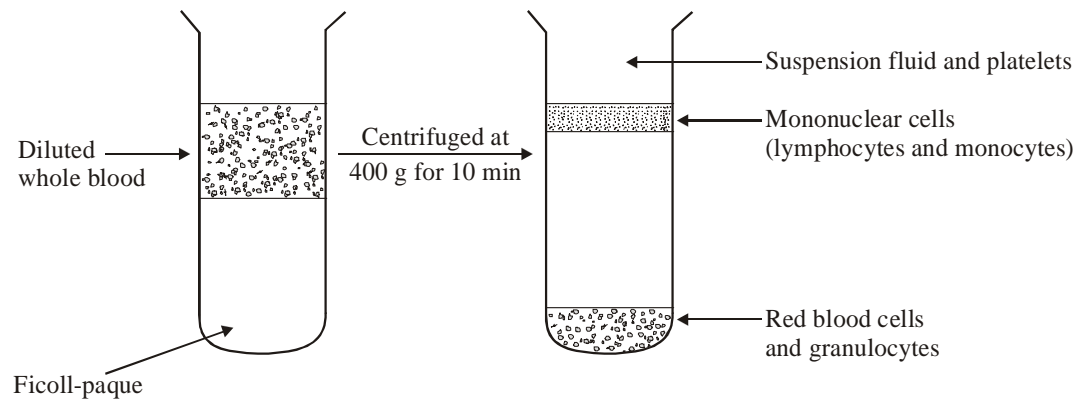


Figure 1: The use of Ficoll-paque to separate lymphocytes from a whole blood.

The upper layer was aspirated with pasteur pipette to within 0.5 cm of the monoclear layer and discarded. The middle layer which was the opaque interface containing the lymphocytes was then aspirated and dispensed into a 15-ml centrifuge tube with care to remove as little of the density gradient as possible. Five (5 ml) of PBS was then added, mixed and centrifuged again at 1,500 rpm for 5 min. to wash the aspirated opaque layer. The supernatant was then decanted and the last drop drained by inverting the tube on top of a paper towel. To each of the tubes containing sedimented white cells was added 0.5 ml of Minimum Essential Medium Eagles (MEM) mixed with foetal calf serum in the ratio of 19:1 and then tapped to resuspend the cells. 0.5 ml of 0.5 % sheep red blood

cell suspension was then pipetted into each of the same tubes and then mixed by shaking gently. The mixture was centrifuged at 1,000 rpm for 5 minutes and gently removed not to disturb the cells. Each tube was then covered with cotton wool, incubated at 37 °C for 10 minutes and then left overnight in a refrigerator at 4°C. A few drops of the wet sediment were gently pipetted and transferred to a microscope slide and covered slowly with cover slip to avoid air bubbles. The rosettes were then viewed microscopically at the objective magnification of X 40. The number of rosetted lymphocytes (those surrounded by three (3) or more red blood cells) and non-rosetted lymphocyte (those free from red blood cells) in the viewed fields were recorded. The percentage of T lymphocytes were then calculated by dividing the number of lymphocytes in rosettes by the total number of lymphocytes (rosetted and non-rosetted) counted.

### **3.9 Pharmacological Studies**

#### *3.9.1 Studies on Central and Peripheral Nervous System*

*Spontaneous Motor Activity (SMA):* The spontaneous motor activity of mice were recorded using ventilated activity cages (LE 886) connected to multi-counter (LE 3806) obtained from LETICA (Spain) using the procedure described by Gamaniel *et al* (1998).

Briefly, adult mice of both sexes with 15.3 – 25.5 g body weight were divided into four groups (n =6). Normal saline (20 ml/kg i.p.) was given to the first group and served as the control. Groups two to four mice received the extract (100, 200, 400 mg/kg i.p.) respectively. 30 min post-treatment, the animals were transferred individually into the LETICA Activity cages. The activity counts were recorded for 6 min after 1 min

latency period, at intervals of 30 min for 120 min. Baseline activity counts were recorded prior to the treatment.

*Test for Exploratory Behaviour in Mice:* The hole-board test of Perez *et al* (1998) was applied in this test. The LETICA board (Signo 720; Printer LE 3333) of 60 cm X 30 cm with 16 evenly spaced holes with in-built infra-red sensors was used for the study.

Adult Swiss albino mice (of either sex; 13.6 – 25.5 g body weight) used for the investigation were placed singly in the arena of the LETICA hole board. The number of times an animal dipped its head into the holes during a 5 min period was automatically counted and recorded by the instrument (Wolfman *et al*, 1994). A baseline count was taken for each mouse.

The mice were then divided into five groups (of 5 mice each). Mice in group one received normal saline (20 ml/kg i.p.) to serve as the negative control. The extract (100, 200, 400 mg/kg) was given intraperitoneally to mice in groups two, three and four respectively, while diazepam (1 mg/kg i.p.) was given to group 5 mice to serve as a reference standard. Recording was repeated as described above at 30, 60 and 90 min post treatment.

*Apomorphine-induced Stereotypic Behaviour:* The effect of the extract on apomorphine-induced stereotypic behaviour was investigated as described by Kenneth and Kenneth (1984). In brief, Swiss albino mice (17.3 – 35.6 g) of both sexes were divided into four groups (n=5). Normal saline (20 ml/kg i.p.) was administered to mice

in group one to serve as the control while graded doses of the extract (100, 200, and 400 mg/kg i.p.) were given to mice in groups two, three and four respectively. 30 min post treatment, apomorphine (0.1 mg/kg i.p.) was administered to each mouse. Signs of stereotypic behaviours, which include mainly sniffing and gnawing were observed and rated. The stereotypic episodes were scored as follows: absence of stereotype (0); occasional sniffing (1); occasional sniffing with occasional gnawing (2); frequent gnawing (3); intense and continuous gnawing (4); intense gnawing and jumping (5). The stereotypic behaviour was measured 1 min post apomorphine administered. They were scored after every minute over 5 min period. The mean of the 5 min period was calculated and recorded.

*Pentobarbitone-induced Sleep (Acute test):* The procedure described by Wambebe *et al* (1985) was adopted for the study. Adult Swiss albino mice of either sex with 15.8 – 32.7 g body weight were divided into five groups (n=5). Normal saline (20 ml/kg i.p.) was given to group one to serve as the control. Groups two to four were treated with the extract (100, 200, 400 mg/kg i.p.) respectively. The fifth group was administered with diazepam (1mg/kg i.p.). 30 min post treatment, pentobarbitone sodium (30 mg/kg i.p.) was administered to each mouse to induce sleep. The time of onset and duration of sleep observed for each mouse was recorded. The criterion for sleep was loss of righting reflex (Miya *et al.*, 1973; Wambebe, 1985; Ramirez *et al.*, 1998). The interval between loss and recovery of righting reflex was used as the index of hypnotic effect (Fujimori, 1965).

This model/test was also adopted as one of the bioassay guides for evaluation of ethylacetate and aqueous fractions of the extract. The study was repeated using 100, 200

and 400 mg/kg i.p. doses of ethylacetate fraction as well as 100, 200 and 400 mg/kg i.p. doses of the aqueous fraction. Diazepam (1 mg/kg i.p.) was used as the reference drug while normal saline (20 ml/kg i.p.) was used as the negative control.

*Use of Pentobarbitone-induced Sleep as a Model to Test Effect of S. bicolor on Microsomal Enzyme of Mice and Rats:* The extract's effect on microsomal enzymes was tested using the pentobarbitone-induced sleep model. Swiss albino mice weighing between 16.1 – 34.7 g of either sex and Wistar rats (81.0 – 172.3 g) of both sexes were used. They were divided into six groups (n=5). Group one received normal saline (20 ml/kg p.o.), Group two received phenobarbitone (1 mg/kg p.o.) to serve as a reference microsomal enzyme inducer drug. Group three was given cimetidine (100 mg/kg p.o.) to serve as reference microsomal enzyme inhibitory drug. Graded doses of the extract (100, 200 and 400 mg/kg p.o.) were administered to groups four, five and six respectively. The treatment was done continuously for six (6) days. 30 min post 6<sup>th</sup> day treatment, pentobarbitone (30 mg/kg i.p. and 40 mg/kg i.p.) were administered to all the groups of mice and rats respectively to induce sleep. Each animal was observed for the onset and duration of sleep, with the criterion for sleep being loss of righting reflex (Miya *et al*, 1973; Wambebe, 1985; Ramirez *et al*, 1998). The index of microsomal enzyme effect was taken as the duration of hypnosis observed in the animals to which it is inversely related.

*Test for Motor Co-ordination (Rota-rod Performance):* The test was conducted following the procedure of Oztwk *et al* (1996). A rota-rod treadmill device (Ugo Basile No. 7650, Varies, Italy) was used to assess the locomotor activity of mice. Adult Swiss albino mice were placed on a horizontal rotating rod with diameter of 5 cm set at 16

revolutions per min. Mice that were able to continuously walk on the rotating rod for 3 min (180 seconds) were selected and grouped into four of five mice each. Mice weighing between 16.4 – 24.7 g and of either sex were used for the study. Normal saline (20 ml/kg i.p.) was given to group one mice to serve as the control. Graded doses of the extract (100, 200, 400 mg/kg i.p.) were administered to mice in groups two, three and four respectively. 30 min. post-treatment, each mouse was placed back on the rotating rod for 180 s (3 min) at intervals of 30 min. for 3 h (180 min). The time an animal fell from the rod within the 180 s was recorded. Failing of an animal more than once to remain on the rod for 180 sec. indicated lack of motor coordination (Fujimori and Cobb, 1965).

### 3.9.2 *Antinociceptive Studies*

*Acetic Acid-induced Writhing Test (Test on Chemical Pain):* The test was done as described by Koster *et al* (1959). Adult Swiss albino mice of either sex weighing 15.1 – 29.1g were used for the investigation. They were grouped into five (n=5). Normal saline (20 ml/kg i.p.) was given to the first group. The second to fourth groups received graded doses of the extract (100, 200, 400 mg/kg i.p.). Acetyl salicylic acid (100 mg/kg i.p.) was administered to the fifth group. At intervals of 30, 60, 90 and 120 min. post administration, 0.75 % glacial acetic acid was administered intraperitoneally to each mouse at the dose of 10 ml/kg. 5 min. after acetic acid injection, the number of writhes made by each mouse within 10 min was counted using a counter. The percent writhes for the treated groups was calculated in relation to the control group. The activity was also expressed as percent inhibition of nociception (reduction in episodes of writhing between saline control and treated groups).

*Tail Flick Test (Test on Mechanical Pain):* The modified techniques of Takagi *et al* (1966) and Huong *et al* (1996) were used. The study involved the use of Ugo Basile Analgesymeter (Cat No. 7200) for the evaluation of force-induced pain stimulus. This instrument exerts a force that increases at a constant rate (a certain number of grams per second). The force is continuously monitored by a pointer moving along a linear scale. Force was applied to tail of each mouse placed on a small plinth under a cone-shaped pusher with a rounded tip. The scale was read at points where each mouse suddenly withdrew its tail. These marked the points at which these mice felt the force-induced pain. This nociceptive response of every mouse was taken prior to treatment to establish the baseline values. Five groups of Swiss albino mice (n=5) of either sex weighing between 15.7 – 31.7 g were used for the study. The first group received normal saline (20 ml/kg i.p.) to serve as the negative control. Mice in groups two to four received the extract (100, 200, 400 mg/kg i.p.). Acetyl salicylic acid (100 mg/kg i.p.) was given to mice in group five. The test was repeated as described above and the nociceptive responses measured every 30 min. over a 120 min. observation period.

*Formalin Test:* The procedure used was that of Dubuisson and Dennis (1977). This procedure shows the possible site(s) of antinociception. Adult Wistar rats of either sex weighing 155.6 – 244.0 g were used. They were grouped into five (of five rats each). Normal saline (20 ml/kg i.p.) was given to group one rats to serve as negative control. The extract (100, 200, 400 mg/kg i.p.) was given to rats in groups two, three and four respectively. Acetylsalicylic acid (100 mg/kg i.p.). was administered to group five rats. 30 min. post treatment, 50 µl (0.05 ml) of 2.5 % formalin was injected under the planter surface of the left hind paw. The rats were then placed in transparent boxes for observation. The severity of pain was recorded as scores: (0), rat walked or stood firmly

on the injected paw; (1) rat partially elevated or favoured the paw; (2), rat elevated the paw from the floor; or (3), rat licked, bit or shook the paw. The cut off points for the observations were every 2 min. For the first 10 min (early phase) and at every 5 min for the period between that 10<sup>th</sup> and 60<sup>th</sup> min (late phase).

### 3.9.3 *Gastrointestinal Studies*

*Intestinal transit Test:* The test was done according to the method of Akah *et al* (1998). Adult Swiss albino mice (15.7 – 25.2 g body weight) of both sexes were used. They were starved of feed for 24 h prior to the experiment but allowed free access to water. The mice were divided into five (n=5). The first group received normal saline (20 ml/kg i.p.) to serve as negative control. Groups two to four mice received the extracts at doses of 100, 200, 400 mg/kg i.p. respectively. Carbachol (1 mg/kg i.p.) was given to the fifth group to serve as the reference standard. 10 min. post treatment, 0.5 ml of a 5 % charcoal suspension in 10 % tragacanth powder was administered orally to each mouse. The mice were sacrificed 30 min later and their abdomen cut open. The percentage of distance traveled by the charcoal plug in the small intestine (from the pylorus to the caecum) was determined for both the treated and control groups (Akah, 1989).

Intestinal transit test was also adopted as one of the bioassay guides. It was repeated on the ethylacetate fraction (100, 200, 400 mg/kg i.p.) and the aqueous fraction (100, 200, 400 mg/kg i.p.) of the leaf extract. Normal saline (20 ml/kg i.p.)-treated group was used as the negative control while atropine (0.1 mg/kg i.p.)-treated group was used as the reference standard.



*Castor Oil-Induced Diarrhoeal Test:* The method of Pulok *et al* (1998) was adopted. Adult Wistar rats (157.9 – 314.0 g) of either sex were used. They were fasted for 18 h. They were then divided into five groups (of five rats each). Normal saline (20 ml/kg i.p.) was given to the first group to serve as the negative control. Graded doses of the extract (100, 200, 400 mg/kg i.p.) were given to groups two to four respectively. Loperamide (10 mg/kg i.p.) was given to the fifth group as a reference standard. 1 h post treatment, 1ml of castor oil was given orally to every rat. They were then observed for defecation. Observation was made over a 4 h period following castor oil administration. The presence of characteristic diarrhoeal droppings were noted on transparent paper surface spread beneath every cage.

*Study of Effect of Extract on Feed and Water Intake After 7 Days of Treatment in Rats:*

This study was carried out on adult Wistar rats of both sexes weighing 130.1 – 203.4 g. They were grouped into six (of five rats each). Group one served as the negative control. Group two rats were given cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.) to serve as reference appetite stimulant. Group three received amphetamine (1.5 mg/kg p.o.) to serve as reference appetite suppressant. Graded doses of the extract (100, 200, 400 mg/kg p.o.) were administered to groups four, five and six respectively. The treatments were given once in the morning at 8.00 h. 30 min post treatment, pre-measured feed and water were given to every group. The animals were allowed access to the feed for only 2 h in the morning (8.30 – 10.30 h) while the water was allowed until the next morning.

In the afternoon, between 14.00 – 16.00 h, another set of pre-measured feed was given to each of the groups. They had access to the feed for 2 h before the feed were

withdrawn. The amount of feed (g) consumed was calculated as the difference between the given feed and the remnant (the feed wasted on the trays were also taken into consideration). The quantity of water consumed over a 24 h period was calculated as the difference between the given volume and remaining volume of water (ml). The treatment and observation lasted for 7 days.

*Studies on Isolated Rabbit Jejunum:* Adult rabbits (2.2 – 2.8 kg) of both sexes used for the study were starved of feed for about 18 h. They were killed with a blow on the head. Each abdomen was cut open and segment of the jejunum (about 2 – 3 cm long) removed and dissected free of adhering mesentery. The tissue was then suspended in 25 ml organ bath containing tyrode solution. The physiological solution constituted of: NaCl 90 g; 10 % KCl 20 ml; 10 %  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  5ml; D-Glucose 10 g;  $\text{NaHCO}_3$  10 g; 10 %  $\text{CaCl}_2$  20 ml;  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  1 ml dissolved in 10 litres of distilled water and maintained at 37 °C and aerated with air.

The effects of acetylcholine and the leaf extract were tested on the strips of jejunum. The responses were recorded isometrically on microdynamometer set at sensitivity of 3.0 mV and speed of 24 mm/min. The concentration of the extract at which it was half maximally effective ( $\text{EC}_{50}$ ) was also determined. This involved the conversion of the log organ bath concentration at which the percent of maximal effect was 50 to normal organ bath concentration.

*Studies on Isolated Guinea Pig Ileum:* Adult guinea pigs (345.0 – 418.0 g) of both sexes used for the study were starved of feed for 18 h. They were killed with a blow on the head and the abdomen of each was cut open. About 2 cm strips of the guinea pig

ileum was removed, the adhering mesentery dissected out. This was mounted in a 25 ml organ bath containing aerated tyrode solution of the above composition and maintained at 37 °C. The set up was connected to microdynamometer recorder set at sensitivity of 2.0 mV and speed of 24 mm/min.

Following equilibration, the effects of histamine, acetylcholine and graded concentrations of the extract were tested on the tissue.

*Studies on Isolated Rat Stomach Fundus Strip:* Adult Wistar rats (152.5 – 210.0 g) of both sexes were used. They were killed with blows on the head. Each abdomen was cut open and the stomach removed. The pyloric region was cut off from the fundus region of the stomach. A strip of the stomach fundus was then made and mounted in aerated Krebs solution constituted of NaCl 69 g; 10 % KCl 35 ml; 10 % Mg SO<sub>4</sub>·7H<sub>2</sub>O 29 ml; 10 % KH<sub>2</sub>PO<sub>4</sub> 16 ml; D-Glucose 20 g; NaHCO<sub>3</sub> 21 g; Molar CaCl<sub>2</sub> 25.2 ml; dissolved in 10 litres of distilled water. The set up was connected to microdynamometer recorder set at sensitivity of 3.0 mV and speed of 24 mm/min.

At equilibration, the effects of acetylcholine histamine, 5-Hydroxytryptamine (5-HT) and the extract were tested on the tissues.

The concentration of the extract at which it was half-maximally effective (EC<sub>50</sub>) was also determined.

#### 3.9.4 *Other Isolated Tissue Studies*

*Studies on Isolated Rat Atria:* Adult Wistar rats of both sexes weighing between 168.3 – 196.0 g were killed with blows on the head. Each thoracic region was opened, and the heart quickly removed and put in Ringer Lorke's solution constituted of NaCl 90 g; 10 % KCl 42 ml; D-Glucose 10 g; NaHCO<sub>3</sub> 5 g; Molar CaCl<sub>2</sub> 10.8 ml; dissolved in 10 litres of distilled water. This was maintained at temperature of 30 °C and aerated with 95 % oxygen and 5 % carbon dioxide mixture. The atria were then carefully removed and suspended in a 25 ml organ bath containing Ringer Lorke's solution.

At equilibration, the effects of adrenaline and graded concentration of the extract were tested on the tissues and recorded on microdynameter recorder set at sensitivity of 10.0 mV and speed of 95 mm/min.

*Studies on Isolated Rat Portal Vein:* Adult Wistar rats of either sex weighing between 172.0 – 183.2 g were used. The rats were killed by a blow on the head and exsanguinated. The abdomen was opened and the portal vein isolated. Each isolated portal vein was suspended in a 25 ml organ bath containing Ringer Lorke's solution maintained at 37 °C and aerated with 95 % oxygen and 5 % carbon dioxide mixture. The tissue was allowed to equilibrate. The spontaneous rhythmic myogenic contractions of the tissue, the effect of adrenaline and the extract on the intrinsic myogenic activity of the portal vein preparation were recorded at a sensitivity of 6 mV and speed of 5 mm/min.

*Studies on Isolated Rat Uterus:* Female Wistar rats weighing 127.3 – 133.5 g were pre-treated with stilboestrol (1 mg/kg s.c.) 48 h before the experiment to induce oestrous.

Each rat was killed by a blow on the head and the abdomen cut open to reveal the fallopian tubes (uterine horns). The horns were dissected free of the adhering tissue. About 2 cm strips was cut out and mounted in a 25 ml organ bath containing De Jalon's solution made of NaCl 90 g; 10 % KCl 42 ml; D-Glucose 15 g; NaHCO<sub>3</sub> 5g; Molar CaCl<sub>2</sub> 2.7 ml; dissolved in 10 litres of distilled water. The solution was maintained at 37 °C and aerated with 95% oxygen / 5% carbon dioxide mixture.

At equilibration, the effects of oxytocin, acetylcholine and graded concentrations of the extract were recorded on microdynamometer set at sensitivity of 2.0 mV and speed of 24 mm/min.

*Studies on Isolated Rat Vas Deferens:* Adult male rats (210.0 – 220.5 g) were stunned and bled. The lower abdomen of each rat was cut open along the midline. The intestine was moved to one side to reveal the vas deferens by the prostate and urethra. The vas deferens on each side was cut at one end and the urethra at the other end. It was dissected off the surrounding tissue and suspended in a 25 ml organ bath containing Krebs's solution maintained at 37 °C and aerated with 95 % oxygen and 5 % carbon dioxide mixture.

At equilibration, the effects of acetylcholine, adrenaline, extract, histamine, atropine and mepyramine were recorded on microdynamometer recorder set at sensitivity of 6.0 mV and speed of 24 mm/min.

### 3.10 Toxicity Studies

#### 3.10.1 *Acute Toxicity Studies*

The modified method of Lorke (1983) was adopted to carry out the study. The method estimates the dose of the extract that will kill 50 % of the animal population ( $LD_{50}$ ). The estimation of  $LD_{50}$  values for the crude extract was done in Swiss albino mice (15.0 – 29.1 g) and Wistar rats (172.6 – 269.0 g) all of either sex. The test routes were both intraperitoneal and oral.

The administration of the extract was done in phases. The first phase involved administration of widely differing doses of the extract (100, 200, 1,500 and 2,000 mg/kg i.p. and p.o.) to determine the range within which toxicity lies. The second phase was dependent on the observations made in the first phase and involved the administration of more specific doses (800, 1,000 and 1,200 mg/kg i.p.) to new sets of experimental mice. The animals treated intraperitoneally and orally were observed for 24 h and 72 h respectively for behavioural effects such as nervousness, ataxia, excitement, alertness, dullness and death.

The intraperitoneal  $LD_{50}$  for the ethylacetate and aqueous fractions of the leaf extract were also evaluated in mice of both sexes, 20.4 – 33.3 g body weight, using doses of 100, 1,000 and 2,000 mg/kg. The mice were observed up to 72 h for any toxicity sign or death.

#### 3.10.2 *Sub-acute Toxicity Studies*

The study was carried out over a 28-day period. Adult male Wistar rats (88.5 - 129.5 g) were used. They were grouped into four (of six rats per group). Groups two to four in

addition to water and feed given *ad libitum* received graded doses of the extract (100, 200 and 400 mg/kg orally) for 28 days. Group one received only water and feed *ad libitum* and served as the control. The first day of dosing was taken as day zero (D0) while the sacrifice day was taken as day twenty eight (D28). The body weight (g) of each rat was taken before commencement of dosing, once every 5 days during the dosing and on the day of sacrifice. The amount of feed and water consumed were measured daily from the quantity of feed and water supplied the previous day and the amount remaining after 24 h. All the animals were observed throughout the four week dosing period for clinical signs/behavioural changes and/or mortality patterns before dosing, during the dosing and up to 4 h after dosing. On D28, all the rats were euthanised and exsanguinated under chloroform anaesthesia. Organs such as heart, liver, lungs, spleen, kidneys and testes were surgically dissected out and weighed in grammes to get the absolute organ weight. The relative organ weight (ROW) of each rat was calculated thus;

$$\text{ROW} = \frac{\text{Absolute Organ Weight (g)}}{\text{Body Weight of rat on sacrifice day (g)}} \times 100$$

Macroscopic/gross examination of the organs were also carried out. All the organs were then fixed in 10 % formal saline for histopathological processing and examinations. Histopathological processing was done using automatic tissue processor (Shandon Citadel model 2000). The steps involved dehydration of the 10 % formal saline-fixed tissues with ethanol to eliminate all the water in the tissues. Clearing was then done with xylene to displace the ethanol. The tissues were then infiltrated with paraffin wax and then embedded for sectioning using rotary microtome. The sectioned tissues were fixed

on slides over a hot plate. They were then stained with hematoxyline and eosin stains and viewed under the microscope at the magnification of X 400.

Blood was collected from the heart of the euthanised rats and a portion dispensed into EDTA- anticoagulant bottles for determination of haematological indices such as PCV, Hb, total WBC and DLC. The remaining blood portion was dispensed into plain tubes and allowed to stand for 3 h to ensure complete clotting. The clotted blood samples were then centrifuged at 3,000 g for 10 min. The clear sera were aspirated and stored frozen for serum biochemical analyses. Biochemical indices were determined using standard ready-to-use kits (Randox Ltd, UK) following the manufacturer's instructions. The indices determined included glutamate oxaloacetate transaminase (GOT) glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), total proteins, albumin, total bilirubin, urea, creatinine, uric acid, total cholesterol and triglycerides.

### **3.11 Statistical Analysis**

The results of the studies were expressed as mean  $\pm$  SEM. The differences between the control and treated means were analysed using one-way or two-way analysis of variance (ANOVA) as appropriate. Student t-test and Least Significant Difference (LSD) were applied where ANOVA showed significant difference. P-values  $< 0.05$  were taken to be statistically significant. Results were presented as tables, diverse charts (histograms, line graphs, etc.) and tracings as appropriate.

### **3.12 Compliance with Good Laboratory Practice (GLP)**

The studies were carried out according to Good Laboratory Practice (GLP) Regulations of Organization for Economic Cooperation and Development – OECD (UNDP/World Bank/WHO, 2001).



#### 4.1 Phytochemical Screening of the Crude Methanolic Extract

The phytochemical analyses carried out on the crude leaf extract showed the presence of saponins, terpenes, steroids and anthraquinones (table 1).

Table 1: Phytochemical constituents of methanolic extract of *S. bicolor* leaf

Chemical Constituents	Test	Inference
Carbohydrates	Molish test	–
Tannins	Ferric chloride test	–
Saponins	Froth test	+
Phlobatannins	General test	–
Phenols	General test	–
Terpenes	Lieberman-Burchard test	+
Steroids	Salkowski's test	+
Anthraquinones	Bortrager's test	+
Flavonoids	Ferric chloride test	–
Alkaloid	Dragendorff's test	–

Absent = –; Present = +

#### 4.2 Haematological Studies

Both increases and decreases were observed at all the tested doses for such parameters as haemoglobin (Hb), Packed cell volume (PCV), total leucocyte count, total red blood cells and bleeding time. However, none of these changes was significantly different

from the control. Increases and decreases were also observed in neutrophil and lymphocyte indices. However, the increase observed for neutrophil was only significant ( $p < 0.05$ ) at the dose of 100 mg/kg p.o. while the decrease observed in lymphocyte was only significantly ( $p < 0.05$ ) different from control at dose of 100 mg/kg p.o. also.

There was a general but non significant decrease in the monocyte count. The basophil and eosinophil counts were just as the control count. A general but non-significant decrease was observed in the clotting time while there was increased platelet count in all the doses. The increase was only significantly ( $p < 0.05$ ) different from control at the dose of 400 mg/kg p.o. (table 2).

Table 2a: Effect of methanolic extract of *S. bicolor* leaf base on some haematological indices of rats treated orally for 14 days

<i>Treatment</i> (p.o. x 14 days)	<i>Hb</i> (g/dL)	<i>PCV</i> (%)	<i>RBC</i> (x 10 <sup>12/L</sup> )	<i>WBC</i> (x 10 <sup>9/L</sup> )	<i>Platelet</i> (x 10 <sup>9/L</sup> )	<i>Bleeding</i> <i>Time</i> (sec)	<i>Clotting</i> <i>Time</i> (sec)
Control	12.72±0.38	38.0 ± 0.9	4.84±0.16	7.78± 0.59	468.0 ± 14.5	31.4±4.1	43.2 ± 2.7
<i>S. bicolor</i>							
100 mg/kg	12.16±0.55	36.4 ± 1.5	4.78± 0.24	8.22 ± 1.00	476.0 ± 17.7	38.0±6.4	38.0 ± 2.5
200 mg/kg	12.86±0.60	38.4 ± 1.7	5.10±0.13	8.28±1.20	491.0 ± 18.7	41.4±6.7	36.4±2.8
400 mg/kg	12.26±0.30	36.6 ± 1.9	4.86±0.29	7.50±1.10	514.8± 18.9*	23.2±3.1	38.4±3.7

Hb = Haemoglobin; PCV= Packed Cell Volume;

WBC = White Blood Cells; DLC= Differential Leucocyte Count;

p.o. = Per os (per oral)

RBC = Red Blood Cells

\*=  $P < 0.05$ , significantly different from control (One-way ANOVA; Student t-test).

Table 2b: Effect of methanolic extract of *S. bicolor* leaf base on differential leucocyte count of rats treated orally for 14 days

<i>Treatment (p.o. x 14 days)</i>	<i>DLC (%)</i>				
	<i>Neu.</i>	<i>Lym.</i>	<i>Mon.</i>	<i>Eos.</i>	<i>Bas.</i>
Control	11.8 ± 1.2	87.8 ± 0.9	0.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
<i>S. bicolor</i>					
100 mg/kg	20.0 ± 4.2*	79.8 ± 2.2*	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
200 mg/kg	19.0 ± 4.3	80.8 ± 2.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
400 mg/kg	11.6 ± 1.6	88.2 ± 1.7	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0

Neu = Neutrophils; Lym= Lymphocytes;  
 Mon= Monocytes; Eos= Eosinophils;  
 Bas = Basophils; DLC= Differential Leucocyte Count

\*= P<0.05, significantly different from control (One-way ANOVA; Student t-test).

### 4.3 Immunomodulatory Studies

#### 4.3.1 Delayed Type Hypersensitivity Reaction (DTH)

There was a general enhancement in the delayed type hypersensitivity reactions of mice in all the groups including the control. This was shown by the increases in the right hind footpad thickness (cm) of mice 24 h, 48 h and 72 h post treatment with a challenging (boost) dose of 0.05 ml of 5 % (v/v) SRBC. However, the only significant (p<0.05) increase was with 400 mg/kg p.o. dose at the 48 h (table 3).

Table 3: Effect of methanolic extract of *S. bicolor* leaf base (100, 200, 400 mg/kg p.o.) on the induration induced by 5 % (v/v) SRBC suspension in mice footpads

Treatment (p.o. x 14 days)	Difference in footpad thickness (cm) post 5 % SRBC challenge					
	24 h		48 h		72 h	
	Induration (cm)	Enhancement (%)	Induration (cm)	Enhancement (%)	Induration (cm)	Enhancement (%)
Normal saline (control; 20 ml/kg)	0.20 ± 0.03	-	0.18 ± 0.04	-	0.14 ± 0.06	-
<i>S. bicolor</i>						
100 mg/kg	0.25 ± 0.02	25.0	0.25 ± 0.02	38.9	0.16 ± 0.03	14.3
200 mg/kg	0.24 ± 0.01	20.0	0.27 ± 0.03	50.0	0.19 ± 0.01	35.7
400 mg/kg	0.25 ± 0.03	25.0	0.31±0.01*	72.2	0.18 ± 0.07	28.6

SRBC = sheep red blood cell

The values for 24, 48, and 72 h represent the difference in the mice foot pad thickness before and after a challenge dose with 0.05 ml of 5 % SRBC.

\* = P < 0.05, significantly different from the control (ANOVA; Student t-test).

#### 4.3.2 Effect of methanolic extract of *S. bicolor* Leaf Base (100 - 400 mg/kg p.o.) on Humoral Responses of Normal and Cyclophosphamide-induced Immuno-deficient Mice

Different degrees of haemagglutination induced by SRBC were observed in all the treatment groups. The antibody titre of the control was 1/8. The haemagglutination at 100 and 200 mg/kg p.o. extract groups were higher than that of the control with the antibody titres of 1/16 each.

However, the sera of 400 mg/kg p.o. extract-treated group showed a higher degree of haemagglutination titre when compared with the other groups. It showed an antibody titre of 1/32. The cyclophosphamide (100 mg/kg p.o.) treated group showed the degree of haemagglutination much smaller than the normal saline treated group with antibody titre of 1/4.

#### 4.3.3 *Clearance Assay for Macrophage Phagocytosis*

Phagocytic index values < 1.0 show no effect (non-active), those between 1.0 –1.5 show slight stimulation of phagocytic rate (active) while those > 1.5 show strong stimulation of phagocytic activity (very active).

The present study revealed that the ratio of the slope of the regression line for extract-treated groups to the slope of the regression line of the control have phagocytic indices of -20.0, -1.8 and 2.8 for 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. doses of *S. bicolor* leaf extract respectively (figure 2; Appendix 1).

#### 4.3.4 *Anti-inflammatory Studies*

Results presented in figure 3; appendix II revealed that intraperitoneal administration of 70 % methanolic leaf extract of *S. bicolor* (100, 200, 400 mg/kg i.p.) did not inhibit fresh egg albumin-induced inflammation (measured as oedema in cm<sup>3</sup>) in rats. Significant increase in oedema was rather recorded at the dose of 400 mg/kg i.p. of the extract. The statistical comparison was done with the normal saline treated group.

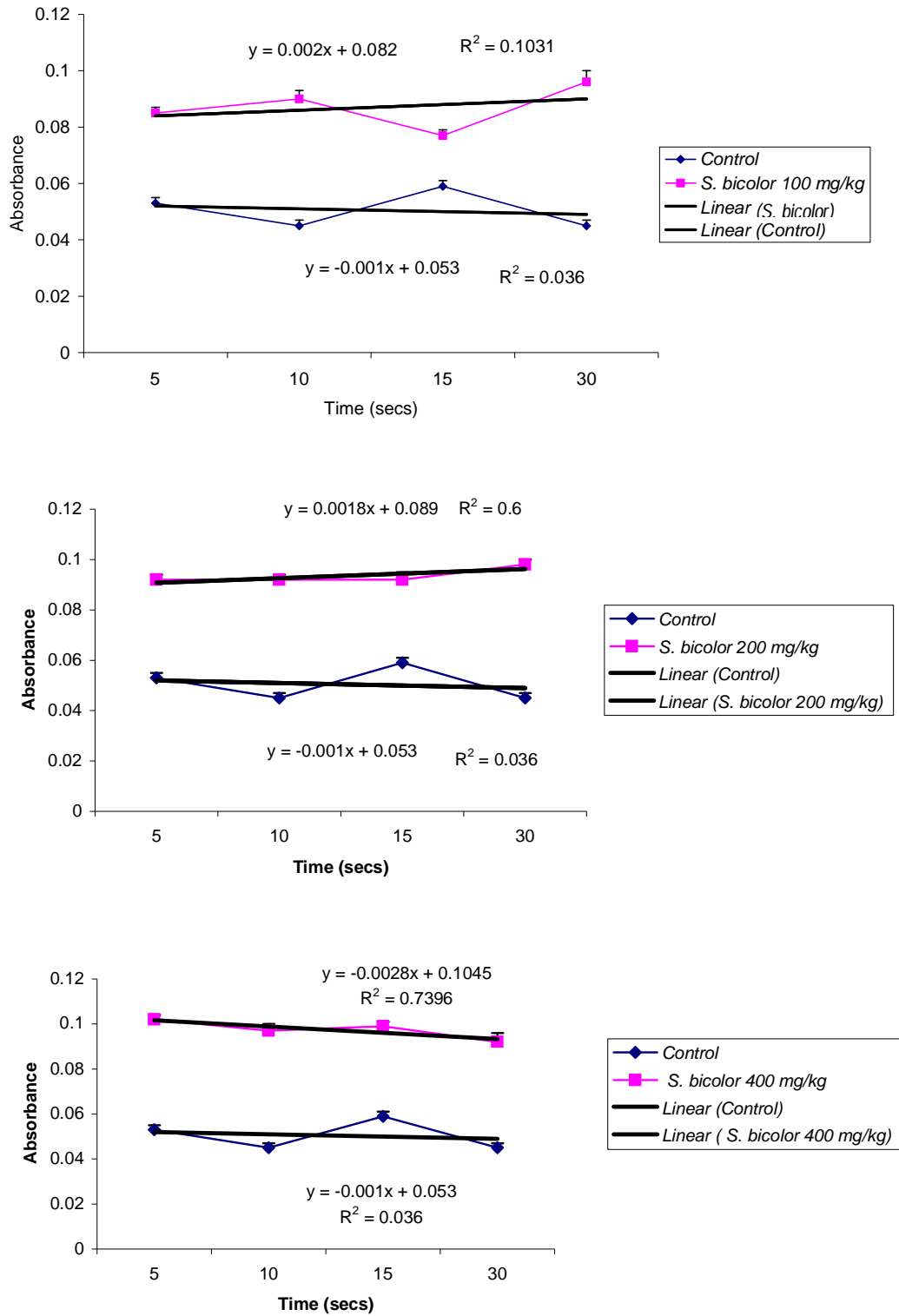


Figure 2: Activated charcoal clearance assay for macrophage phagocytosis of rats treated with methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.; n = 5).

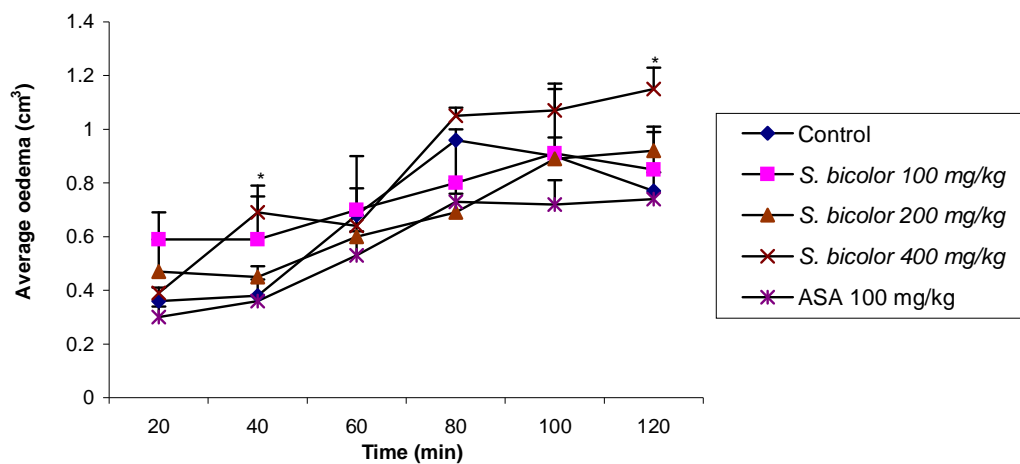


Figure 3: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on fresh egg albumin-induced paw oedema in rats.

\* =  $P < 0.05$ ; statistically different between treated and control group (two-way ANOVA; Student t-test,  $n = 5$ )

-Data derived from appendix II-

#### 4.3.5 Estimation of T-lymphocytes by 'E' Rosettes

Decreases in the number of rosetted cells were observed in the *S. bicolor*-treated groups. The leaf extract at the graded doses of 100, 200 and 400 mg/kg p.o. showed rosetted cell percentages of 33.1, 20.2 and 37.3 respectively while that of normal saline treated group was 38.0. This decrease was only significant ( $p < 0.05$ ) at the dose of 200 mg/kg p.o. (figure 4, Appendix III).

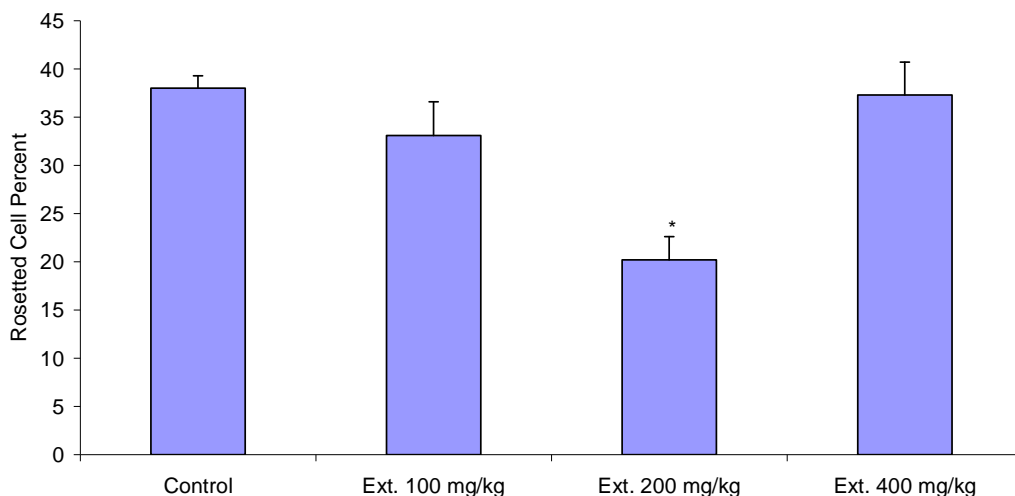


Figure 4: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on rat lymphocyte-sheep red blood cell rosetting

\*= P<0.05; statistical difference between treated and control group (one-way ANOVA; Student t-test, n = 5).

-Data derived from appendix III-

#### 4.4 Pharmacological Studies

##### 4.4.1 Spontaneous Motor Activity (SMA)

*S. bicolor* leaf extract (100, 200 and 400 mg/kg i.p.) produced a significant ( $p < 0.05$ ) reduction of spontaneous motor activity in mice in relation to the control at any time interval. This effect was time related. Maximum effect was observed at 60th minute for 100 mg/kg dose and at 90th minute for both 200 and 400 mg/kg doses. The SMA of the control group was also observed to have decreased from the 60th minute of the experiment. The reduction was however, not as the treated groups (figure5; Appendix IV).



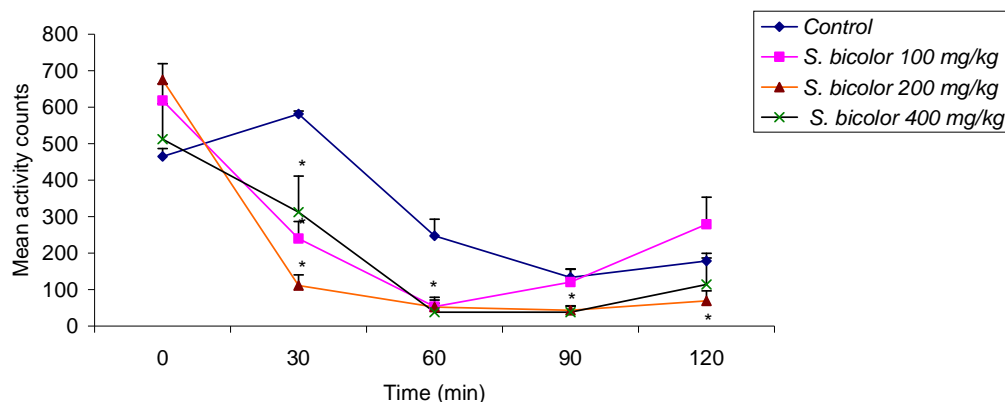


Figure 5: Effect of methanolic extract of *S. bicolor* leaf base (100, 200 and 400 mg/kg i.p.) on spontaneous motor activity in mice.

\*=  $P < 0.05$ ; statistical difference between treated and control group (one-way ANOVA; Student t-test,  $n = 5$ ).

-Data derived from appendix IV-

#### 4.4.2 Exploratory Behaviour

The study revealed a reduction in the exploratory activity of mice in all the groups. However, the observed reductions were more in the treated groups. The statistical comparisons made for every group with its zero reading showed statistical significance ( $p < 0.05$ ) for 100 mg/kg group of extract at 30, 60 and 90 min, for extract (200 mg/kg) group only at 90 min, 400 mg/kg group of the extract at 30, 60 and 90 min and for diazepam (1 mg/kg i.p.) at 30, 60 and 90 min.

The statistical comparisons made between the treated groups and normal saline control group showed significant ( $p < 0.05$ ) reduction for the extract at 30 min for 100 mg/kg i.p. dose, at 90 min for 400 mg/kg i.p. dose and for diazepam at 30 and 60 min intervals (table 4).

Table 4: Effect of methanolic extract of *S. bicolor* leaf base (100, 200, 400 mg/kg i.p.) on exploratory behaviour of mice

<i>Treatment (i.p.)</i>	<i>Mean Head-dips ± SEM</i>			
	<i>0 min</i>	<i>30 min</i>	<i>60 min</i>	<i>90 min</i>
Normal saline (control; 20 ml/kg)	79.8 ±6.3	70.6 ±14.1	59.8 ±13.3	43.2 ±14.4
<i>S. bicolor</i>				
100 mg/kg	81.0 ± 11.3	30.4 ± 9.3* <sup>@</sup>	46.8 ±9.2*	40.4 ±4.6*
200 mg/kg	66.4 ±5.1	78.4 ±22.5	47.6 ±10.9	25.4 ±9.7*
400 mg/kg	91.4 ±16.8	61.0 ±12.6*	39.2 ±10.5*	16.4 ±4.4* <sup>@</sup>
Diazepam (1 mg/kg)	70.4 ±7.6	39.4 ±14.0* <sup>@</sup>	17.0 ±6.3*	16.8 ±4.7*

SEM= Standard error of mean; 0 min = Before treatment;

\*=  $p < 0.05$ , statically different from zero reading of same group (2-way ANOVA; Least Significant Difference - LSD)

<sup>@</sup> =  $P < 0.05$ , statistical difference from normal saline control reading (2-way ANOVA; LSD).

#### 4.4.3 *Apomorphine-induced Stereotypic Behaviour*

The methanolic leaf base extract of *S. bicolor* (100 – 400 mg/kg i.p.) did not inhibit apomorphine-induced stereotypic behaviour in mice (table 5).

Table 5: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on apomorphine (0.1 mg/kg i.p.)-induced stereotypic behaviour in mice

<i>Treatment</i>	<i>Mean score per 5 min ± SEM</i>
Control	2.76 ± 0.9
<i>S. bicolor</i>	
100 mg/kg i.p.	3.10 ± 1.0
200 mg/kg i.p.	4.40 ± 0.7
400 mg/kg i.p.	4.60 ± 0.6

#### 4.4.4 *Pentobarbitone-induced Sleep (Acute Test)*

The methanolic leaf base extract of *S. bicolor* caused a reduction in the sleep onset of mice dosed once with the extract. This effect was not dose-dependent and was only significant ( $p < 0.05$ ) at the dose of 100 mg/kg i.p. The extract also prolonged the duration of pentobarbitone sleep in a manner that was also not dose-dependent. The prolongation was only significant ( $p < 0.05$ ) at the dose of 100 mg/kg i.p. Diazepam (1 mg/kg i.p.) on the other hand significantly ( $p < 0.05$ ) caused a reduction in the sleep onset and prolongation of the sleep duration (table 6).

However, the aqueous (100 – 400 mg/kg i.p.) and ethylacetate (100 – 400 mg/kg i.p.) fractions of *S. bicolor* leaf extract produced no significant effect on both the onset and duration of pentobarbital-induced sleep in mice while diazepam (1 mg/kg i.p.) caused a significant ( $p < 0.05$ ) prolongation of sleep duration (table 7).

Table 6: Effect of methanolic extract of *S. bicolor* leaf base (100 - 400 mg/kg i.p.) on pentobarbitone-induced sleep in mice

<i>Treatment (i.p.)</i>	<i>Onset of sleep (min)</i>	<i>Duration of sleep (min)</i>
Normal saline (control; 20 ml/kg)	5.6 ± 0.51	56.0 ± 5.1
<i>S. bicolor</i>		
100 mg/kg	4.2 ± 0.37*	92.0 ± 9.3*
200 mg/kg	5.4 ± 0.70	69.2 ± 13.2
400 mg/kg	4.0 ± 0.71*	111.3 ± 43.1*
Diazepam (1 mg/kg)	3.2 ± 0.60*	126.0 ± 16.7*

Values are expressed as mean ± SEM (n=5)

\* = p < 0.05; statistical difference between treated and control group (ANOVA, Student t-test)

#### 4.4.5 Pentobarbitone-induced Model for Microsomal Enzyme of Mice and Rats

Mice dosed cumulatively for six days with the extract had slightly increased onset of pentobarbitone (30 mg/kg i.p.)-induced sleep at 100 mg/kg p.o. dose of the extract while the doses of 200 and 400 mg/kg p.o. of the extract produced reduced sleep onset. These observations were however non-significantly different from the control.

Table 7: Effects of aqueous and ethylacetate fractions of *S bicolor* leaf extract (100 - 400 mg/kg i.p.) on pentobarbitone-induced sleep in mice

<i>Treatment (i.p.)</i>	<i>Onset of sleep (min)</i>	<i>Duration of sleep (min)</i>
Normal saline (control; 20 ml/kg)	6.0 ± 1.5	87.0 ± 22.3
Aqueous fraction		
100 mg/kg	3.8 ± 0.5	109.0 ± 37.3
200 mg/kg	4.0 ± 1.1	94.0 ± 37.4
400 mg/kg	7.3 ± 2.0	89.8 ± 24.2
Ethylacetate fraction		
100 mg/kg	8.5 ± 0.3	80.0 ± 24.7
200 mg/kg	4.3 ± 0.8	101.5 ± 32.4
400 mg/kg	7.0 ± 1.3	88.8 ± 19.1
Diazepam (1 mg/kg)	4.0 ± 0.4	137.0 ± 6.7*

Values are expressed as mean ± SEM

\* = p< 0.05; statistical difference between treated and control group (ANOVA, Student t-test)

The duration of pentobarbitone sleep was slightly reduced by the extract at 100 mg/kg p.o. dose while they were prolonged at 200 and 400 mg/kg p.o. doses. These effects were also non-significantly different from the control. Phenobarbitone (1 mg/kg p.o.) produced non-significant reduction of sleep onset but a significant (p<0.05) reduction in the duration of sleep while cimetidine (100 mg/kg p.o.) produced significant (p<0.05) prolongation of duration of pentobarbitone-induced sleep in mice (table 8).

Conversely, rats treated cumulatively for six days with the methanolic extract had a slightly reduced onset of pentobarbitone (40 mg/kg i.p.)-induced sleep at 100 mg/kg dose while the doses of 200 and 400 mg/kg p.o. slightly increased the sleep onset time. These effects were non-significantly different from the control. However, the duration of pentobarbitone sleep was prolonged by all the doses of the extract (100 – 400 mg/kg p.o.). These effects were not dose-dependent and was only significant at dose of 400 mg/kg p.o. Phenobarbitone (1 mg/kg p.o.) produced some reduction in onset and duration of pentobarbitone-induced sleep while cimetidine (100 mg/kg p.o.) produced a significant ( $p < 0.05$ ) prolongation of the sleep (table 9)

Table 8: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on microsomal enzyme of mice tested on pentobarbitone-induced sleep model

<i>Treatment (p.o. x 6 days)</i>	<i>Onset of sleep (min)</i>	<i>Duration of sleep (min)</i>
Control (normal saline; 20 ml/kg)	15.25 ± 7.0	31.25 ± 5.7
<i>S. bicolor</i>		
100 mg/kg	18.42 ± 6.4	28.00 ± 1.2
200 mg/kg	6.83 ± 0.5	38.5 ± 0.4
400 mg/kg	13.00 ± 0.5	72.75 ± 27.0
Phenobarbitone (1 mg/kg)	11.33 ± 2.6	10.00 ± 0.6*
Cimetidine (100 mg/kg)	3.47 ± 0.46*	92.23 ± 16.0*

Values are expressed as mean ± SEM; Pentobarbitone dose = 30 mg/kg i.p.

\*=  $p < 0.05$ ; statistical difference between treated and control group (ANOVA, Student t-test).

Table 9: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on microsomal enzyme of rats tested on pentobarbitone-induced sleep model

<i>Treatment (p.o. X 6 days)</i>	<i>Onset of sleep (min)</i>	<i>Duration of sleep (min)</i>
Normal saline (control; 20 ml/kg)	3.75 ± 0.25	90.5 ± 7.27
<i>S. bicolor</i>		
100 mg/kg	3.33 ± 0.33	105.67 ± 4.33
200 mg/kg	4.25 ± 0.75	128.75 ± 39.1
400 mg/kg	4.50 ± 0.50	128.50 ± 10.0*
Phenobarbitone (1 mg/kg)	3.25 ± 0.25	86.25 ± 3.42
Cimetidine (100 mg/kg)	3.67 ± 0.33	222.0 ± 19.17*

Values are expressed as mean ± SEM; pentobarbitone dose = 40 mg/kg i.p.  
 \*= p<0.05; statistical difference between treated and control group (ANOVA, Student t-test).

#### 4.4.6 Test for Motor Co-ordination (Rota-rod Performance)

The methanolic leaf base extract of *S. bicolor* (100 – 400 mg/kg i.p.) did not produce significant effect on the rota-rod performance of the mice. Most of the mice were able to stay on the rod through the 180 s (3 min) cut-off time point without falling (table 10).

#### 4.4.7 Antinociceptive Studies

*Acetic Acid-induced Writhing Test:* The methanolic leaf base extract of *S. bicolor* significantly (p<0.05) reduced the number of acetic acid-induced abdominal constrictions in mice at all the tested doses (100, 200 and 400 mg/kg i.p.). The antinociceptive effect was dose-dependent having a dose-pain reduction effects calculated to be 47.6 %, 70.7 % and 76.4 % for 100, 200 and 400 mg/kg doses

respectively. The effect occurred throughout the observation period of 120 min. The maximal antinociceptive effect for 100 mg/kg dose was at 120 min having writhing of 29.9 % (equivalence of pain inhibition of 70.1 %). The maximal antinociceptive effect for 200 mg/kg dose was at 90 min with percent writhes of 11.8 % (equivalence pain inhibition of 88.2 %) while that of 400 mg/kg dose was at 60 min with writhes of 10.5 % (pain inhibition equivalence of 89.5 %). The result was comparable to that of aspirin (100 mg/kg i.p.; figure 6; Appendix V).

Table 10: Effect of methanolic extract of *S. bicolor* leaf base. (100 – 400 mg/kg i.p.) on motor coordination (rota-rod performance) of mice

Treatment (i.p.)	Time (second)					
	30	60	90	120	150	180
Normal saline						
(control; 20 ml/kg)	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0
<i>S. bicolor</i>						
100 mg/kg	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0
200 mg/kg	180.0±0.0	176.6±3.4	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0
400 mg/kg	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0	180.0±0.0

Values are expressed as mean ± SEM (n=5)



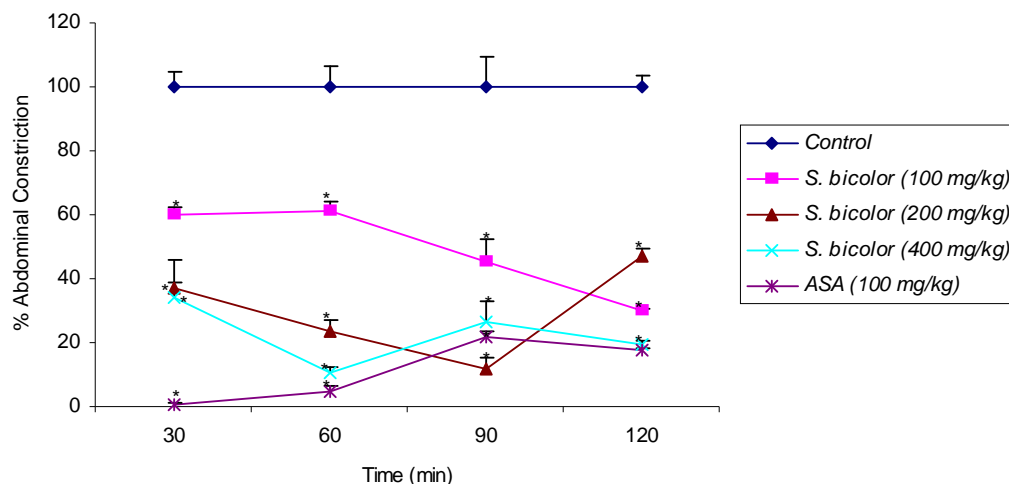


Figure 6: Effect of methanolic extract of *S. bicolor* leaf base.(100 – 400 mg/kg i.p.) on glacial acetic acid-induced abdominal constriction in mice.

\* P<0.05; Statistical difference from control (Least Significant Difference – LSD, n = 5)

-Data derived from appendix V-

*Tail Flick Test:* The methanolic leaf base extract of *S. bicolor* (100 – 400 mg/kg i.p.) generally increased the ability of mice to withstand force-induced pain (mechanical pain). The antinociceptive effect was not dose-dependent and occurred at all the observation time intervals. However, the effect at 100 mg/kg dose was not significantly different from the control at all the time intervals, while the effect at 400 mg/kg dose was significant at 120 min. The effect at 200 mg/kg dose was significantly ( $p < 0.05$ ) different from the control at 60, 90 and 120 min intervals. The results compared favourably with ASA (100 mg/kg i.p., figure 7a; Appendix VI).

The aqueous fraction (100 – 400 mg/kg i.p.) of *S bicolor* leaf base extract also increased the ability of mice to withstand mechanical pain. The aqueous fraction at the tested

doses of 100, 200 and 400 mg/kg i.p. produced significant ( $p < 0.05$ ) antinociceptive effect in comparison with the zero minute (0 min) reading of the mice. The result is comparable to that of ASA (100 mg/kg i.p.).

Ethylacetate fraction on the other hand did not show antinociceptive effect when compared with the mice zero minute (0 min) reading (figure 7b; Appendix VII).

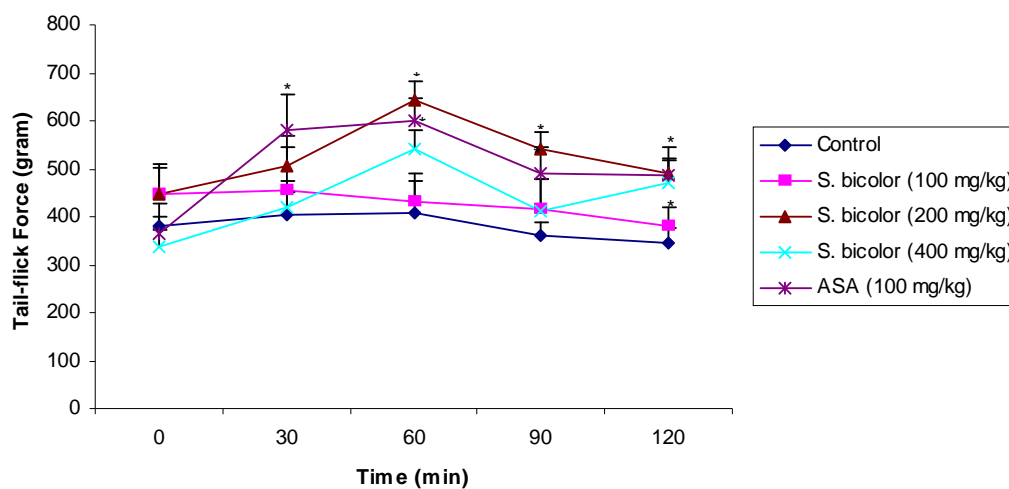


Figure 7a: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on force-induced pain in mice.

\* =  $P < 0.05$ ; statistical difference from control (ANOVA, Student t-test)

-Data derived from appendix VI-

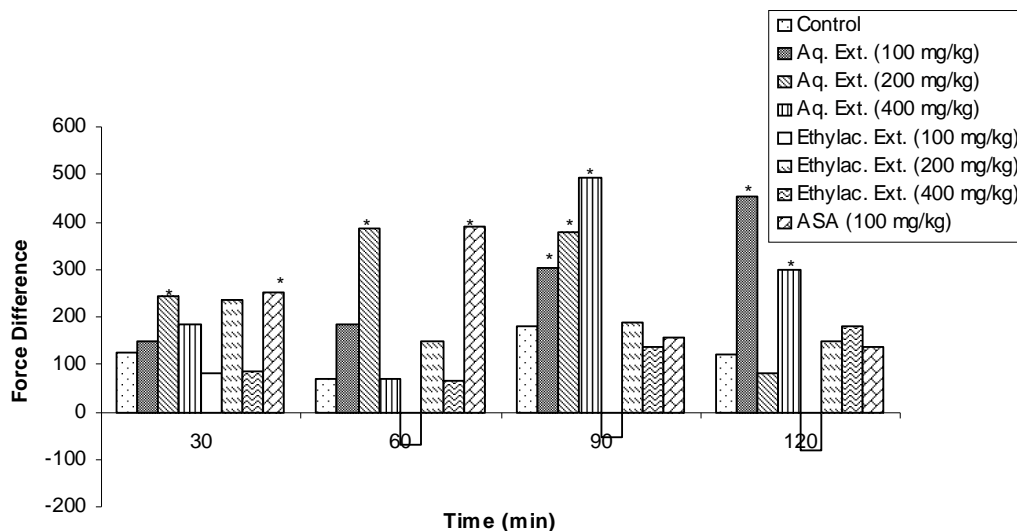


Figure 7b: Effect of the aqueous and ethylacetate fractions (100 – 400 mg/kg i.p.) of *S. bicolor* leaf extract on force-induced pain in mice.

\* =  $P < 0.05$ ; statistical difference from control (ANOVA, Student t-test)  
Ext. = Extract; Aq. = Aqueous; Ethylac. = Ethylacetate

Note: The plotted force values were the differences between the force exerted at zero minute and all the time intervals.

-Data derived from appendix VII-

*Formalin Test:* The methanolic extract dose-dependently reduced formalin-induced pain in both early and late phases of the experiment. However, the percent pain inhibition between 0 – 10 min were 10.2 %, 38.8 % and 55.1 % for 100, 200 and 400 mg/kg i.p. doses of the extract respectively, while the percent pain inhibition between 15 – 60 min were 5 %, 32.9 % and 37.9 % respectively for 100, 200 and 400 mg/kg i.p. doses of the extract. This was unlike ASA (150 mg/kg i.p.) that showed higher percent pain inhibition of 81.4 % in the late phase (15 – 60 min) and only 24.5 % in the early phase (0 – 10 min; table 11).

Table 11: Effect of methanolic extract of *S. bicolor* leaf base (100, 200, 400 mg/kg i.p.) on early and late phases of formalin-induced pain in rats

<i>Treatment</i>	<i>Early phase</i>		<i>Late phase</i>	
	<i>Score of pain</i>	<i>% pain inhibition</i>	<i>Score of pain</i>	<i>% pain inhibition</i>
Control	1.96 ± 0.6	-	2.80 ± 0.3	-
<i>S. bicolor</i>				
100 mg/kg i.p.	1.76 ± 0.6	10.2	2.66 ± 1.2	5.0
200 mg/kg i.p.	1.20 ± 0.6	38.8	1.88 ± 0.5	32.9
400 mg/kg i.p.	0.88 ± 0.5	55.1	1.74 ± 0.6	37.9
ASA (150 mg/kg i.p.)	1.48 ± 0.4	24.5	0.52 ± 0.4*	81.4

\*p<0.05; statistical difference from control (Student t-test).

#### 4.4.8 *Effect on Intestinal Transit*

The methanolic leaf base extract of *S. bicolor* (100 – 400 mg/kg i.p.) significantly (p<0.05) decreased the propulsive movement of charcoal meal through the gastrointestinal tract when compared with the normal saline control. The observed effects were dose-dependent with percent charcoal movement of 19.2 %, 8.6 % and 6.6 %, equivalence of inhibitory percentages of 80.8 %, 91.4 % and 93.4 % for 100, 200 and 400 mg/kg i.p. respectively. Carbachol (1 mg/kg i.p.) on the other hand significantly (p<0.05) increased the intestinal propulsion with inhibitory percentage of 21.3 % equivalent to 78.7 % charcoal movement (table 12).

Table 12: Inhibitory effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on intestinal motility in mice

<i>Treatment</i>	<i>Mean body weight (g)</i>	<i>Mean Intestinal length (cm)</i>	<i>Mean distance travelled by charcoal (cm)</i>	<i>Movement of charcoal as % of intestinal length (%)</i>
Control	19.82 ± 1.3	37.96 ± 1.1	14.30 ± 0.9	37.7
<i>S. bicolor</i>				
100 mg/kg i.p.	20.30 ± 1.3	35.78 ± 1.1	6.86 ± 1.1*	19.2
200 mg/kg i.p.	19.14 ± 1.5	34.76 ± 1.7	3.00 ± 1.5*	8.6
400 mg/kg i.p.	19.20 ± 0.8	36.60 ± 2.2	2.42 ± 0.7*	6.6
Carbachol (1 mg/kg i.p.)	22.63 ± 0.6	35.63 ± 1.2	28.03 ± 4.5**	78.7

\*= p<0.05; significant reduction in intestinal propulsion;

\*\*= p<0.05; significant increase in intestinal propulsion (one-way ANOVA, Student t-test).

The aqueous fraction of the 70 % v/v methanolic extract of *S. bicolor* leaf (100 – 400 mg/kg i.p.) also reduced charcoal meal movement at all the tested doses when compared with the normal saline control. The doses of 100, 200 and 400 mg/kg i.p. produced percent charcoal movement of 42.7, 46.3 and 50.5 respectively. This is equivalent to propulsion inhibitory percentages of 57.3 %, 53.7 % and 49.5 % respectively. The inhibitory effect decreased dose-dependently and was only significant at the dose of 100 mg/kg i.p. The highest inhibitory effect was also observed at this dose.

The ethylacetate fraction (100 – 400 mg/kg i.p.) also reduced charcoal meal movement at all the tested doses with percent charcoal movement of 36.9, 37.0 and 23.0 (equivalent to propulsion inhibitory percentages of 63.1 %, 63.0 % and 77.0 %) for doses of 100, 200 and 400 mg/kg i.p. respectively). The effect was not dose-dependent

but was significant ( $p < 0.05$ ) at all the tested doses. The dose of 400 mg/kg i.p. produced the highest inhibitory effect.

Atropine (0.1 mg/kg i.p.) also reduced charcoal movement in comparison with the control, having inhibitory percentage of 26.4 % (equivalent to 73.6 % charcoal movement). However, the inhibitory effects of the aqueous and ethylacetate fractions of the extract were much higher than that of the tested dose of atropine (table 13).

Table 13: Inhibitory effects of the aqueous and ethylacetate fractions of *S. bicolor* leaf extract (100 – 400 mg/kg i.p.) on intestinal motility in mice

<i>Treatment</i>	<i>Mean body weight (g)</i>	<i>Mean intestinal length (cm)</i>	<i>Mean distance travelled by charcoal (cm)</i>	<i>Movement of charcoal as percentage of intestinal length</i>
Control	28.63 ± 3.2	31.98 ± 3.0	24.78 ± 2.9	77.5
Aqueous fraction				
100 mg/kg i.p.	26.18 ± 3.7	31.83 ± 2.1	13.60 ± 1.9	42.7*
200 mg/kg i.p.	26.45 ± 3.3	38.60 ± 0.7	17.88 ± 3.9	46.3
400 mg/kg i.p.	26.98 ± 3.2	30.70 ± 2.8	15.5 ± 6.3	50.5
Ethylacetate fraction				
100 mg/kg i.p.	28.43 ± 1.4	40.10 ± 2.1	14.78 ± 1.1	36.9*
200 mg/kg i.p.	29.00 ± 1.3	37.23 ± 2.0	13.78 ± 1.9	37.0*
400 mg/kg i.p.	27.08 ± 2.5	41.10 ± 2.8	9.45 ± 2.9	23.0*
Atropine (0.1 mg/kg i.p.)	25.75 ± 4.4	36.65 ± 0.6	26.98 ± 2.5	73.6

\* =  $p < 0.05$ ; Significant reduction in intestinal propulsion (one way ANOVA; Student t-test, n = 5).

#### 4.4.9 *Effect on Castor Oil-induced Diarrhoea*

The methanolic leaf base extract of *S. bicolor* at the dose of 100 mg/kg i.p. produced an effect similar to that of normal saline control group with 0.0 % castor oil-induced diarrhoeal inhibition. On the other hand, the doses of 200 and 400 mg/kg i.p. of the extract exhibited marked anti-diarrhoeal activity with 100 % diarrhoeal inhibition. This effect compared favourably with standard anti-diarrhoeal drug, loperamide (10 mg/kg i.p.). The diarrhoeal onset time observed in the 100 mg/kg extract group was shorter than that of the normal saline control group (table 14).

Table 14: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on castor oil-induced diarrhoea in rats

<i>Treatment</i>	<i>Mean diarrhoeal Onset time (min)</i>	<i>Diarrhoeal score</i>				<i>Inhibition (%)</i>
		++	+	-	Total score	
Control	174.3 ± 25.6	3	1	0	7	-
<i>S. bicolor</i>						
100 mg/kg i.p.	158.0 ± 25.8	2	3	-	7	0.0
200 mg/kg i.p.	0.0 ± 0.0	-	-	5	0*	100.0
400 mg/kg i.p.	0.0 ± 0.0	-	-	5	0*	100.0
Loperamide (10 mg/kg i.p.)	0.0 ± 0.0	-	-	5	0*	100.0

\* =  $p < 0.05$ ; Statistical difference from control (student t-test)

Note: (++) = 2; + = 1; - = 0 multiplied by the number of animals with a particular severity of diarrhoea).

#### 4.4.10 *Effect of Methanolic Extract of S. bicolor leaf base on Feed and Water Intake After 7-days Treatment in Rats*

For the morning feed intake, amphetamine (1.5 mg/kg p.o.) produced a significant ( $p < 0.05$ ) decrease in feed intake throughout the 7-day treatment. On the other hand, the

extract at all the tested doses (100 – 400 mg/kg p.o.) and cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.) produced both increases and decreases in the feed intake. The increases were significant ( $p < 0.05$ ) for cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.) on day one (D1), extract (100 mg/kg p.o.) on days one and seven (D1 and D7), extract (200 mg/kg p.o.) on days one, two, six and seven (D1, D2, D6, D7) and extract (400 mg/kg p.o.) on days one and six (D1, D6). The decreases in morning feed intake were significant ( $p < 0.05$ ) for cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.) on days three and four (D3, D4), for extract (100 mg/kg p.o.) on days three, four, five and six (D3, D4, D5, D6), for extract (200 mg/kg p.o.) on day five (D5) and for extract (400 mg/kg p.o.) on day three (D3; table 15). Thus, the effects of the extract and the appetite stimulant on morning feed intake were not consistent.

For the afternoon feed intake, both increases and decreases were recorded but none of the treatments (cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.); amphetamine, 1.5 mg/kg or *S. bicolor* leaf extract, 100 – 400 mg/kg p.o.) produced any significant effect (table 16).

Amphetamine (1.5 mg/kg p.o.) significantly ( $p < 0.05$ ) reduced the rat water intake throughout the 7-day treatment while cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.) and *S. bicolor* leaf extract (100 – 400 mg/kg p.o.) caused both increases and decreases in rat water intake. The decreases were significant for cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.) on day 1 (D1), *S. bicolor* extract (100 mg/kg p.o.) on days one, two and four (D1, D2, D4), extract (200 mg/kg p.o.) on days one, two and four (D1, D2, D4) and extract (400 mg/kg p.o.) on days one, four (D1, D4). The increases in water intake were



significant for cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.) on day five (D5), for extract (100 mg/kg p.o.) on day three (D3), extract (200 mg/kg p.o.) on day six (D6) and extract (400 mg/kg p.o.) on days three and six (D3, D6; table 17). Again, like for feed intake, effects of the extract and the appetite stimulant were not consistent.

Table 15: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on morning feed intake of rats during 7-day treatment

<i>Treatment</i>	<i>Feed intake (g/150 g rat/h)</i>						
	<i>Day 1</i>	<i>Day2</i>	<i>Day 3</i>	<i>Day 4</i>	<i>Day 5</i>	<i>Day 6</i>	<i>Day 7</i>
Control	4.1	5.2	5.8	5.0	4.3	4.5	5.0
<i>S. bicolor</i>							
100 mg/kg p.o.	4.8*	5.5	4.9**	3.3**	2.9**	3.6**	5.8*
200 mg/kg p.o.	5.4*	6.5*	5.6	4.9	3.1**	5.3*	6.4*
400 mg/kg p.o.	5.9*	5.4	4.9**	4.6	4.1	5.5*	5.6
Cyproheptadine $\alpha$ - ketoglutarate (0.3 mg/kg p.o.)	4.9*	5.7	4.4**	3.7**	3.8	3.9	5.1
Amphetamine (1.5 mg/kg p.o.)	2.1**	2.3**	2.5**	2.4**	2.3**	3.3**	2.6**

\*=Significant increase; \*\*= significant decrease from the control at  $p < 0.05$ ; ANOVA and Least Significant Difference (LSD).

Note: Treatment was done once in the morning daily (X 7 days).

Table 16: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on afternoon feed intake of rats during 7-day treatment

<i>Treatment</i>	<i>Feed intake (g/150 g rat/h)</i>						
	<i>Day 1</i>	<i>Day 2</i>	<i>Day 3</i>	<i>Day 4</i>	<i>Day 5</i>	<i>Day 6</i>	<i>Day 7</i>
Control	6.3	1.2	1.9	2.9	2.5	3.7	4.3
<i>S. bicolor</i>							
100 mg/kg p.o.	4.0	2.7	3.2	4.0	3.9	3.8	6.4
200 mg/kg p.o.	2.8	2.7	2.8	4.2	3.4	3.1	5.1
400 mg/kg p.o.	4.2	3.9	4.0	4.5	3.3	5.0	5.2
Cyproheptadine $\alpha$ -ketoglutarate (0.3 mg/kg p.o.)	4.3	3.3	3.4	4.1	2.9	3.6	5.7
Amphetamine (1.5 mg/kg p.o.)	4.7	3.6	3.7	4.2	3.4	4.9	4.8

Note: Observations were based on the treatment given once in the morning of each day (X 7 days).

Table 17: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on water intake of rats during 7-day treatment

<i>Treatment</i>	<i>Water intake (ml/150 g rat/24 h)</i>						
	<i>Day 1</i>	<i>Day 2</i>	<i>Day 3</i>	<i>Day 4</i>	<i>Day 5</i>	<i>Day 6</i>	<i>Day 7</i>
Control	28.2	28.2	25.9	28.2	23.5	25.9	25.9
<i>S. bicolor</i>							
100 mg/kg p.o.	21.8**	24.2**	32.4*	24.2**	24.2	26.6	26.6
200 mg/kg p.o.	21.4**	23.8**	25.7	26.1**	23.8	28.5*	26.2
400 mg/kg p.o.	26.8**	29.2	29.2*	26.8**	24.3	31.6*	28.0
Cyproheptadine $\alpha$ - ketoglutarate (0.3 mg/kg p.o.)	25.7**	28.1	25.7	28.1	28.1*	26.2	25.7
Amphetamine (1.5 mg/kg p.o.)	19.0**	19.0**	23.8**	23.8**	19.0**	112.5**	112.5**

\*=Significant increase; \*\*=Significant decrease from the control at  $p < 0.05$ ; ANOVA and Least Significant Difference (LSD).

#### 4.4.11 Effect of Methanolic Extract of *S. bicolor* leaf base on Isolated Rabbit Jejunum

The methanolic extract of *S. bicolor* leaf base (0.04 – 2.56 mg/ml) produced a concentration-dependent relaxation of rabbit jejunum. This effect was in contrast to those of acetylcholine (0.004 – 0.016  $\mu\text{g/ml}$ ) and histamine (0.4 – 0.8  $\mu\text{g/ml}$ ), which caused contraction of the same tissues (figure 8; Appendices VIII and IX). The organ bath concentration of the extract at which it is half-maximally effective ( $\text{EC}_{50}$ ) on rabbit

jejunum was 0.21 mg/ml (an equivalence of log organ bath concentration of  $-0.67$ ; figure 8, Appendices VIII and IX).

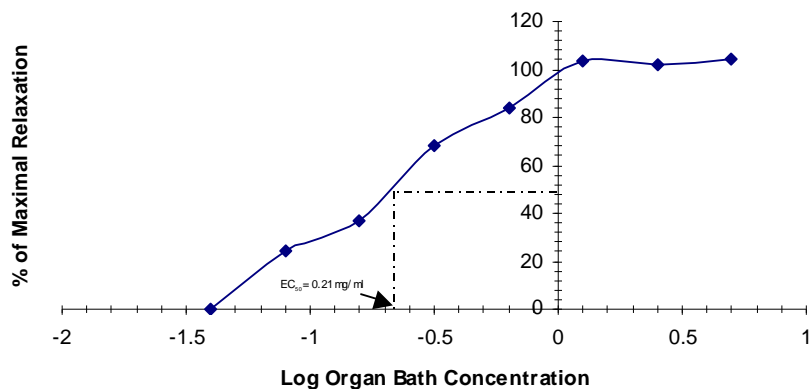


Figure 8: Log concentration-dependent relaxation of rabbit jejunum by 70 % methanolic leaf base extract of *S. bicolor* (0.04 - 5.12 mg/ml)

-Data derived from appendix IX-

#### 4.4.12 Effect of Methanolic Extract of *S. bicolor* leaf base on Guinea Pig Ileum

The methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) did not produce any effect on smooth muscles of guinea pig ileum except in one out of four preparations studied in which a slight relaxation was observed at extract concentrations of 1.28 – 5.12 mg/ml. Histamine (0.04 – 0.16  $\mu\text{g/ml}$ ) and acetylcholine (0.02 – 0.16  $\mu\text{g/ml}$ ) produced contraction of all the studied tissues (Appendices X and XI).

#### 4.4.13 Effect of Methanolic Extract of *S. bicolor* leaf base on Isolated Rat Stomach Fundus Strip

The methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) contracted the smooth muscles of rat stomach fundus strip. The effect was not exactly concentration-dependent. Acetylcholine (0.04 – 0.16  $\mu\text{g/ml}$ ), histamine (0.08 – 0.16  $\mu\text{g/ml}$ ) and 5-hydroxy tryptamine (5-HT; 0.004 – 0.016  $\mu\text{g/ml}$ ) also contracted these tissues but in a concentration dependent manner (figure 9, Appendices XII and XIII). The methanolic extract at the tested organ bath concentrations (0.04 – 5.12 mg/ml) produced percent of maximal contractions greater than 50 % on isolated rat stomach fundus strip.

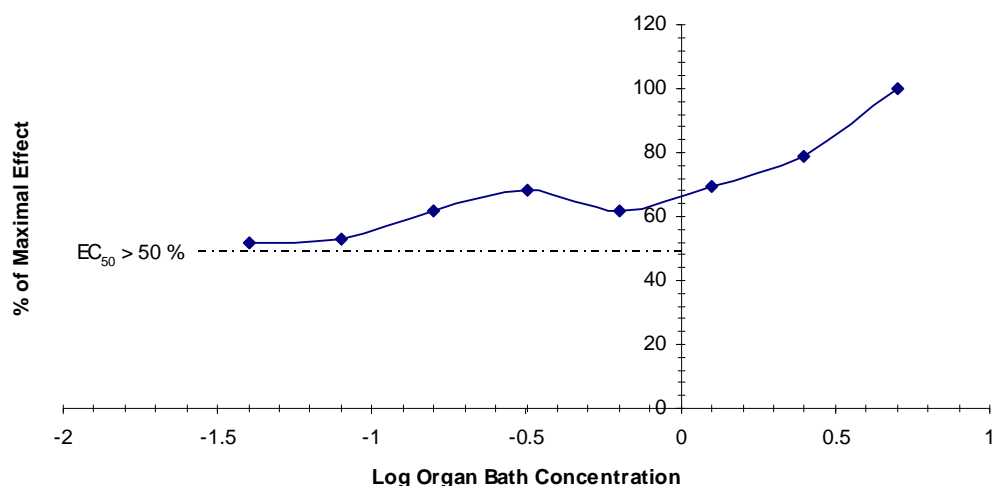


Figure 9: Contraction effect of methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) on rat stomach fundus strip

-Data derived from appendix XIII-

Note: Higher and lower concentrations of the extract will be needed to produce effects lower than 50 % for subsequent calculation of the concentration of the extract at which it is half-maximally effective ( $\text{EC}_{50}$ ).

#### 4.4.14 *Effect of Methanolic Extract of S. bicolor leaf base on Isolated Rat Atria*

The methanolic extract of *S. bicolor* leaf base (0.4 – 3.2 mg/ml) produced no effect on rat atria. However, adrenaline (0.04 µg/ml) increased the contractile amplitude of these tissues (Appendix XIV).

#### 4.4.15 *Effect of Methanolic Extract of S. bicolor leaf base on Isolated Rat Portal Vein*

The methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) did not alter the intrinsic myogenic contraction of rat portal vein. Adrenaline (0.04 – 0.16 µg/ml) on the other hand increased the contractile amplitude of these tissues (Appendix XV).

#### 4.4.16 *Effect of Methanolic Extract of S. bicolor leaf base on Isolated Rat Uterus*

The methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) did not produce any effect on stilboestrol pre-treated uteri. Oxytocin (0.004 – 0.016 µg/ml) and acetylcholine (0.02 – 0.08 µg/ml) contracted these tissues in a concentration- dependent manner (Appendix XVI).

The contractile effects of oxytocin ((0.008 – 0.016 µg/ml) were not blocked by the extract (0.64 – 2.56 mg/ml; Appendix XVII).

#### 4.4.17 *Effect of Methanolic Extract of S. bicolor leaf base on Isolated Rat Vas Deferens*

The methanolic extract of *S. bicolor* leaf base (0.4 – 25.6 mg/ml) caused minimal contractions on rat vas deferens. These contractions occurred at all the tested

concentrations but they were not concentration-dependent. Acetylcholine (0.04 – 0.16 µg/ml) produced a concentration-dependent contraction of the tissues. The contractile amplitude of acetylcholine at these concentrations were not high. They were however higher than those of the extract at all the tested concentrations (Appendix XVIII).

Atropine (0.4 µg/ml) blocked the contractile effect of acetylcholine (0.16 µg/ml) on the tissue but could not block the contractile effect of the methanolic extract of *S. bicolor* leaf base (3.2 mg/ml) on same tissue (Appendix XIX).

The contractile effects of acetylcholine (0.16 µg/ml) and the methanolic extract of *S. bicolor* leaf base (3.2 mg/ml) were not blocked by mepyramine (0.16 µg/ml; Appendix XX).

## 4.5 Toxicity Studies

### 4.5.1 Acute Toxicity Studies

No overt toxicity sign or death was observed in rats and mice 72 h post oral treatment with 100 – 2,000 mg/kg doses of the methanolic extract of *S. bicolor* leaf base. The estimated oral median lethal dose (LD<sub>50</sub>) of the extract in rats and mice was therefore > 2000 mg/kg p.o.

The rats treated intraperitoneally (i.p.) with the methanolic leaf extract (100 – 2,000 mg/kg) showed no overt toxicity sign or death 24 h post treatment. However, all the rats treated with 2,000 mg/kg i.p. dose became recumbent and died within 48 h of the intraperitoneal treatment while those treated with 100 – 1,000 mg/kg i.p. doses neither showed toxicity signs nor death 72 h post i.p. treatment.

For the estimation of the intraperitoneal median lethal dose (LD<sub>50</sub> i.p.) in rats, assessment based on 24 h post treatment showed a median lethal dose (LD<sub>50</sub>) > 2000 mg/kg i.p. since no overt toxicity sign or death was observed in i.p.-treated rats after 24 h.

However, an assessment based on 48 h post intraperitoneal treatment observation gave a calculated median lethal dose of 1,414.2 mg/kg i.p. in rats.

The mice treated with doses of the methanolic extract ≤ 1,200 mg/kg i.p. showed neither toxicity signs nor death 24 h post treatment. At the dose of 1,500 mg/kg i.p., the mice were calm, dull with increased respiratory rate. At this dose, mortality of 66.7 % and 100.0 % occurred within 24 h and 48 h of i.p. treatment respectively.

The mice treated intraperitoneally with 2,000 mg/kg dose were calm, dull, recumbent with increased respiratory rate. A mortality of 100.0 % occurred at this dose within 24 h. The estimated intraperitoneal median lethal dose in mice was 1,248.0 mg/kg i.p. and 1,341.6 mg/kg i.p. for 24 h and 48 h post treatment respectively.

For the ethylacetate fraction of *S. bicolor* leaf extract, 66.7 % and 33.3 % of 1,000 and 2,000 mg/kg i.p.-treated mice were dull, immobilized with increased respiration within 12 min post administration. All the mice later recovered and no further toxicity signs or death was observed 24 h, 48 h and 72 h post intraperitoneal administration. The intraperitoneal LD<sub>50</sub> of ethylacetate fraction of *S. bicolor* leaf extract in mice was therefore > 2,000 mg/kg.



For the aqueous fraction of *S. bicolor* leaf extract, only 33.3 % of mice treated intraperitoneally with the dose of 2,000 mg/kg were dull, immobilized with increased respiration within 10 min of administration. The mice also recovered and no further toxicity sign or death was observed 24 h, 48 h and 72 h post i.p. administration. The intraperitoneal LD<sub>50</sub> of aqueous fraction of *S. bicolor* leaf extract in mice was therefore > 2,000 mg/kg.

#### 4.5.2 *Sub-acute Toxicity Studies*

*Clinical Signs and Mortality Patterns:* Throughout the treatment duration of twenty eight (28) days, no adverse clinical sign or toxicity sign was observed in all the rats. No mortality was recorded in all the groups.

*Effect on Body Weight, Feed and Water Consumption Rate:* Increase in body weight was observed throughout the study duration in all the groups (including the control group). However, the methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) did not produce any significant change in the body weight of treated rats when compared with the control (table 18).

Feed were consumed variably throughout the treatment period in all the groups. Both increases and decreases in feed consumption were recorded. However, non of these changes differed significantly from the control (table 19).

Table 18: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on body weight of male rats treated for 28 days

<i>Treatment</i>	<i>Mean Body Weight (g)</i>						
	<i>D0</i>	<i>D5</i>	<i>D10</i>	<i>D15</i>	<i>D20</i>	<i>D25</i>	<i>D28</i>
Control	99.90 ± 3.9	104.40 ± 4.2	107.73 ± 4.7	112.58 ± 5.7	119.7 ± 6.7	127.95 ± 7.6	131.12 ± 9.0
<i>S. bicolor</i>							
100 mg/kg p.o.	101.92 ± 5.8	104.93 ± 4.1	107.33 ± 3.9	112.40 ± 4.3	119.95 ± 5.3	128.43 ± 5.7	132.08 ± 6.9
200 mg/kg p.o.	101.50 ± 6.0	103.4 ± 2.4	106.67 ± 4.6	109.95 ± 4.9	115.98 ± 5.2	123.48 ± 6.2	130.12 ± 6.7
400 mg/kg p.o.	100.00 ± 6.3	103.06 ± 6.1	107.78 ± 7.0	112.03 ± 7.5	118.08 ± 8.5	126.07 ± 9.0	131.35 ± 9.4

D0 – D28 = treatment days

Table 19: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on feed intake of male rats treated for 28 days

<i>Treatment</i>	<i>Mean feed intake (g)</i>			
	<i>Week 1</i>	<i>Week 2</i>	<i>Week 3</i>	<i>Week 4</i>
Control	57.19 ± 2.7	56.69 ± 2.6	68.81 ± 4.1	80.4 ± 4.6
<i>S. bicolor</i>				
100 mg/kg p.o.	60.69 ± 1.8	54.93 ± 2.7	66.49 ± 4.3	73.48 ± 3.6
200 mg/kg p.o.	51.73 ± 1.8	52.31 ± 2.2	64.36 ± 4.1	80.04 ± 3.3
400 mg/kg p.o.	51.04 ± 2.1	54.37 ± 2.1	65.54 ± 3.1	89.24 ± 3.7

Water was also taken variably with both increases and decreases in consumption recorded in all the groups throughout the 28 day treatment period. The only significant intake was the increase recorded in week 2 for 100 mg/kg p.o. treated rats (table 20).

Table 20: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on water intake of male rats treated for 28 days

<i>Treatment</i>	<i>Mean water intake (ml)</i>			
	<i>Week 1</i>	<i>Week 2</i>	<i>Week 3</i>	<i>Week 4</i>
Control	96.07 ± 5.1	101.79 ± 5.5	96.43 ± 6.4	85.42 ± 3.8
<i>S. bicolor</i>				
100 mg/kg p.o.	105.36 ± 5.8	116.00 ± 3.9*	99.93 ± 6.6	93.75 ± 5.4
200 mg/kg p.o.	99.29 ± 4.8	107.14 ± 4.0	92.86 ± 4.0	87.50 ± 3.2
400 mg/kg p.o.	101.79 ± 4.6	105.36 ± 5.8	82.14 ± 5.0	79.17 ± 5.3

\*=p<0.05, significant increase in water intake; Student t-test.

*Effect on Relative Organ Weight:* There were no significant changes in the relative weight of the liver, heart, spleen and lungs. However, 200 mg/kg dose produced a significant (p<0.05) reduction in the relative weight of the kidneys while 400 mg/kg dose caused a significant increase in the relative weight of the testes (table 21).

Table 21: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on relative organ weight of male rats treated for 28 days

<i>Treatment</i>	<i>Mean Relative Organ Weight (g/100 g)</i>					
	<i>Liver</i>	<i>Kidney</i>	<i>Heart</i>	<i>Spleen</i>	<i>Lungs</i>	<i>Testes</i>
Control	3.74 ± 0.10	0.61 ± 0.03	0.37 ± 0.03	0.47 ± 0.06	0.73 ± 0.10	1.36 ± 0.05
<i>S. bicolor</i>						
100 mg/kg p.o.	3.48 ± 0.10	0.60 ± 0.02	0.34 ± 0.01	0.43 ± 0.05	0.65 ± 0.03	1.44 ± 0.06
200 mg/kg p.o.	3.65 ± 0.05	0.47 ± 0.05*	0.33 ± 0.02	0.48 ± 0.03	0.68 ± 0.07	1.55 ± 0.09
400 mg/kg p.o.	3.43 ± 0.09	0.63 ± 0.02	0.33 ± 0.01	0.46 ± 0.03	0.69 ± 0.04	1.51 ± 0.03**

\*=Significant reduction; \*\*= significant increase in relative organ weight (p<0.05;

ANOVA; Student t-test).

*Gross and Histopathological Observations:* No gross abnormality was seen in the morphologies/features, consistencies and appearances of the liver, kidney, heart, spleen, lungs and testes of the male rats treated for 28 days with the methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.).

Histopathological examination of organs stained with hematoxylin and eosin stains, viewed under microscope at X 400 magnification revealed that there were no abnormalities in the kidneys (plate 3), heart (plate 4), spleen (plate 5) and lungs (plate 6) of the male rats treated for 28 days with the methanolic extract of *S. bicolor* leaf base. No abnormality was also seen in the liver at the dose of 100 mg/kg. However, some nuclear enlargements and fragmentations, a few collapsed blood vessels and some cellular infiltrations were seen in the liver at doses of 200 and 400 mg/kg (plate 7). Testes showed mild architectural distortion. Some seemingly fibrotic tissues were detected only at doses of 200 and 400 mg/kg. Some of these features were also seen in some of the control slides, possibly showing they may not really be abnormalities due to the extract (plate 8).

**Plate 3:** Kidney sections of rat treated with methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) for 28 days (magnification X 400 H and E stain)

**Plate 4:** Heart sections of rat treated with methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) for 28 days (magnification X 400 H and E stain)

**Plate 5:** Spleen sections of rat treated with methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) for 28 days (magnification X 400 H and E stain)

**Plate 6:** Lung sections of rat treated with methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) for 28 days (magnification X 400 H and E stain)



**Plate 7:** Liver sections of rat treated with methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) for 28 days (magnification X 400 H and E stain)

**Plate 8:** Testes sections of rat treated with methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) for 28 days (magnification X 400 H and E stain)

*Haematological Effects:* The result showed both increases and decreases in the total white blood cells. The lymphocytes were dose-dependently increased while monocytes, eosinophils and basophils were virtually absent in all the viewed fields. A reduction was recorded for the neutrophils, haemoglobin and packed cell volume. However, none of these changes (increases or decreases) differed significantly from the control (table 22).

Table 22: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on haematological indices of male rats treated for 28 days

Treatment	PCV (%)	Hb (g/dl)	Total WBC ( $\times 10^9/l$ )	DLC				
				Neut.	Lymph.	Mono.	Eos.	Bas.
Control	38.00 $\pm$ 2.2	12.72 $\pm$ 0.7	8.45 $\pm$ 1.2	23.33 $\pm$ 5.0	76.67 $\pm$ 5.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>S. bicolor</i>								
100 mg/kg p.o.	35.67 $\pm$ 2.1	11.90 $\pm$ 0.7	9.80 $\pm$ 1.2	17.67 $\pm$ 2.6	80.50 $\pm$ 3.4	0.17 $\pm$ 0.17	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
200 mg/kg p.o.	34.67 $\pm$ 2.5	11.57 $\pm$ 0.8	9.93 $\pm$ 2.5	17.17 $\pm$ 2.3	81.17 $\pm$ 3.3	$\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
400 mg/kg p.o.	34.17 $\pm$ 1.9	11.45 $\pm$ 0.6	8.23 $\pm$ 0.9	16.83 $\pm$ 3.4	83.17 $\pm$ 3.4	0.00 $\pm$ 0.0	0.00 $\pm$ 0.0	0.00 $\pm$ 0.0

PCV = Packed cell volume; Hb = haemoglobin; WBC = White blood cell; DLC = differential leucocyte count; Neut = neutrophils; Lymph = lymphocytes; mono = Monocytes; Eos. Eosinophils; Bas. = basophils.

*Effects on Hepatic Function Indices:* Both increases and decreases were recorded for direct bilirubin, total bilirubin and albumin. Increases were recorded for glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase while reductions were recorded for total protein. However, these effects were neither dose-dependent nor significantly different from the control (table 23).

Table 23: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on hepatic function indices of male rats treated for 28 days

<i>Treatment</i>	<i>GOT</i> (IU/L)	<i>GPT</i> (IU/L)	<i>Direct</i> <i>bilirubin</i> (mg/dl)	<i>Total</i> <i>Bilirubin</i> (mg/dl)	<i>Alkaline</i> <i>phosphatase</i> (IU/L)	<i>Albumin</i> (mg/dl)	<i>Total</i> <i>protein</i> (mg/dl)
Control	65.0 ± 6.4	28.50 ± 1.4	0.11 ± 0.05	0.49 ± 0.09	109.65 ± 11.1	3.13 ± 0.1	6.57 ± 0.3
<i>S. bicolor</i>							
100 mg/kg p.o.	70.33 ± 6.0	35.67 ± 6.5	0.16 ± 0.03	0.38 ± 0.1	113.5 ± 10.7	3.43 ± 0.08	6.50 ± 0.3
200 mg/kg p.o.	68.33 ± 7.0	30.17 ± 1.8	0.04 ± 0.01	0.43 ± 0.09	119.78 ± 8.2	3.00 ± 0.09	6.02 ± 0.11
400 mg/kg p.o.	69.33 ± 5.3	35.5 ± 3.6	0.18 ± 0.06	0.57 ± 0.1	112.8 ± 8.6	3.37 ± 0.04	6.35 ± 0.1

GOT= Glutamate oxaloacetate transaminase

GPT= Glutamate pyruvate transaminase

*Effects on Renal Function Indices:* There were both decreases and increases in all the renal function indices. However, the changes in urea and creatinine were not significantly different from the control at all the tested doses, while the doses of 100 and 200 mg/kg p.o. of the extract significantly ( $p < 0.05$ ) reduced uric acid (table 24).

*Effects on Serum Lipid Profile:* Cholesterol had both reductions and an increase but none of these changes differed significantly from the control. On the other hand, triglyceride was generally reduced at all the tested doses. The reduction was only significant ( $p < 0.05$ ) at the dose of 200 mg/kg p.o. (table 25).

Table 24: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on renal function indices of male rats treated for 28 days

<i>Treatment</i>	<i>Urea (mg/dl)</i>	<i>Creatinine (mg/dl)</i>	<i>Uric acid (mg/dl)</i>
Control	26.43 ± 5.9	0.50 ± 0.08	5.08 ± 0.35
<i>S. bicolor</i>			
100 mg/kg p.o.	33.90 ± 6.4	0.40 ± 0.05	4.10 ± 0.02*
200 mg/kg p.o.	19.72 ± 5.5	0.52 ± 0.07	4.13 ± 0.40*
400 mg/kg p.o.	27.18 ± 6.5	0.45 ± 0.07	5.58 ± 0.67

\* = P < 0.05 (significant reduction; Student t-test)

Table 25: Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on serum lipid profile of male rats treated for 28 days.

<i>Treatment</i>	<i>Triglyceride (mg/dl)</i>	<i>Cholesterol (mg/dl)</i>
Control	115.07 ± 5.7	102.58 ± 9.3
<i>S. bicolor</i>		
100 mg/kg p.o.	106.63 ± 10.3	103.40 ± 7.3
200 mg/kg p.o.	92.37 ± 7.0*	94.52 ± 7.7
400 mg/kg p.o.	104.28 ± 9.3	101.47 ± 12.4

\* = p < 0.05 (significant reduction; Student t-test).

This thesis has shown an overview of the effect of 70 % v/v methanolic leaf base extract of *S. bicolor* on different test models. One of the goals of this study was to establish the haematological and immune potentiating functions of the extract of the leaf base of *Sorghum bicolor* in experimental animals. The immunological models adopted in the study took into consideration, the specific and non-specific kinds of immunity.

The study has successfully evaluated the extract for haematological and body defence (immunomodulatory) properties, part of which it is ethnomedicinally used.

The study evaluated the effect of the extract on haematological indices after 14-day treatment and after 28-day sub-acute toxicity treatment and revealed that the extract (100 – 400 mg/kg p.o.) showed no significant effect on the Hb, PCV, total leucocyte count, total red blood cell, bleeding time, monocytes, basophil, eosinophil, clotting time of rats treated for 14 days. The neutrophils and platelets of the 14-day treated rats were however significantly increased at 100 mg/kg p.o. and 400 mg/kg p.o. doses respectively, while their lymphocytes were significantly decreased at 100 mg/kg p.o. The extract did not produce any significant effect on all the tested haematological parameters (TLC, lymphocytes, monocytes, eosinophils, basophils, neutrophils, Hb, PCV) of rats treated for 28 days.

Blood cell production (haemopoiesis) in adult occurs in the bone marrow, spleen, liver and lymphatic tissue including the thymus; these comprise the reticuloendothelial system. The non-significant effect of the extract on most of the haematological indices

could therefore mean that these blood cell production sites were not made haemopoietically active by the extract. The significant ( $P < 0.05$ ) increase in neutrophil observed only at 100 mg/kg p.o. after 14 days treatment was non-significantly decreased after 28 days treatment with the extract. This could be because neutrophils are usually increased in acute conditions and are usually short-lived (Baker *et al* 1985). This could be the reason for the initial increase after 14 days treatment and subsequent decrease after 28 days treatment. On the other hand, the lymphocyte count was significantly ( $P < 0.05$ ) decreased after 14-day treatment, dose dependently but non-significantly increased after 28 days of treatment. This could be because increase in lymphocytes (lymphocytosis) is a common feature in chronic conditions usually associated to persistence of foreign material that mobilizes immunologic reaction (Ward, 1978); Baker *et al.*, 1985). The persistence/continuous administration of the extract for 28 days may have therefore contributed to the increase in lymphocyte count, though non-significantly.

The results also showed that platelet was increased at all the treated doses of 100 – 400 mg/kg. p.o. after 14 days treatment with the extract. The increase was significant at 400 mg/kg p.o. dose.

On contact with collagen, exposed when blood vessels are injured, platelets (thrombocytes) rapidly adhere to the damaged vessels and with one another to form a platelet plug. During this process, the soluble blood coagulation factors are activated to produce a mesh of insoluble fibrin around the clumped platelets. This assists and strengthens the platelet plug and produces a blood clot, which prevents further blood loss (Baker *et al*, 1985). This probably explains the decrease (though non-significant)

clotting time recorded in the study. It may also justify the ethnomedicinal use (though the inflorescence) of the plant as haemostatic. It is possible that constituents responsible for this haemostatic effect are present in both the inflorescence and the leaves.

However, an increase in platelet (thrombocytosis) has been reported to be of clinical importance, not just because it predisposes to thrombocytosis but also because it can be associated with haemorrhage, surgery and fractures of bones (Baker *et al*, 1985). It can arise in the course of chronic infections and cancer (Livingstone, 1987). The thrombocytosis observed in the study was probably due to long time (chronic) administration of the extract or haemorrhagic activity in the cause of the treatment. This will further be clarified in subsequent studies.

It is worth noting however, that the results of the haematological investigation did not corroborate the ethnomedicinal use of the plant leaf portions for stimulation of blood production.

Immunomodulation is one of the most important alternatives in the control of diseases with additional advantages of amplifying the specific responses to vaccines. Immunomodulatory compounds also offer the prospect of reversing immunosuppression caused by stress, infection, surgery or environmental pollution (Chauhan, 1998) and possibly HIV.

The immunomodulatory investigations carried out on *S. bicolor* leaf base extract showed that delayed-type hypersensitivity reaction (which was used to assess the effect of the extract on cell-mediated immunity) was measured as the degree of the induration



induced by 5 % v/v suspension of SRBC in mice foot pads. The result revealed an increase in the footpad induration of all the mice. This is suggestive of enhanced chemotactic activity of the extract.

According to Luo *et al* (1995) and Chong *et al* (1998), SRBC is a T-dependent antigen. The presence of an antigen enables presensitised small-to-medium T lymphocytes to become activated, and then produce and release physiologically active substances into the surrounding area. These effectors cause accumulation of macrophages, monocytes, polymorphonuclear cells and probably other non-sensitised leucocytes into the area of antigen contact. The unsensitised cells are transformed into blast cells, which mature to produce and release more physiologically active materials. Thus, the initial effect is amplified (hence, the induration) until the invading antigen is destroyed (Zink, 1980; Sen *et al*, 1992). This natural event probably explains why mice in all the groups, including the control had increased footpad induration. The extract-treated groups however, had higher degrees of induration suggestive of the extract's ability to particularly enhance the activation of more T-lymphocytes or cause the mobilization of tissue macrophages at the site of antigen re-exposure (challenge). It is possible that the extract acted as phagocytic cell attractant (chemotactic effect).

Since the ability of an immunostimulant to restore an impaired (suppressed) immune system is an important criterion for its potential use, experimental animal model using cyclosporine A, corticoids or cytostatic agents as immune suppressors should also be established (Wagner and Jurcic, 1991). Immunosuppressive drug is one that can attenuate the expression of at least one type of immune response and cyclophosphamide (which was used to test the effect of the extract on humoral response) has been

classified as cytotoxic immunosuppressant with a mechanism aimed at inhibiting nucleic acid synthesis to thwart the stimuli for proliferation of the lymphocytes involved in immune response (Stewart, 1980). The result of the investigation showed antibody titres higher than those of the negative control and the cyclophosphamide-treated group. This was however not significant. This suggests a weak effect on humoral response and could mean that the B-lymphocytes were not strongly sensitized.

Macrophages play important role in nonspecific and specific immune responses. In innate immunity, the phagocytosis of foreign materials by macrophages and other phagocytes facilitate the effector function of these cells in homeostasis, host defence and inflammation. In acquired immunity, macrophages and other phagocytes contribute to regulation of both humoral and cellular immune responses.

Macrophages recognize and process foreign materials and then present them to B-and T-lymphocytes. Together with cytotoxic T-lymphocytes and natural killer cells, macrophages served as effector cells to provide immune surveillance against tumor cells. In addition to these cell-to-cell interactions, macrophages also influence the immune system by secretion of cytokines in both an autocrine and paracrine manner to protect the host against infectious agents, tumor cell and toxic agents as well as to modulate the behaviour of cells in the environment of triggered cells (Dhuley, 1997; Yesilada *et al*, 1998).

The rate of clearance of carbon from blood by macrophages is governed by an exponential equation. This seems to be the general way in which inert particulate matter is cleared from the blood (Damre *et al*, 2003). In this study, activated charcoal was used

as the inert particulate matter and the result showed no activity by *S. bicolor* leaf base extract on macrophage phagocytosis at doses of 100 and 200 mg/kg p.o. while the dose of 400 mg/kg p.o. showed strong stimulation of phagocytic rate. It is therefore possible that the extract at the high dose of 400 mg/kg p.o. primarily activated macrophages which in turn secrete cytokines to stimulate other immunocytes like neutrophils. This has been suggested for similar plant activities by Thalte and Dahanukar (1989) and Praveen Kumar *et al* (1994).

This could therefore account for the increase in the neutrophils observed in the haematology study. The significant rise in activated charcoal clearance by extract at 400 mg/kg p.o. dose could also be indicative of stimulation of the reticuloendothelial system as was suggested by Pallabi *et al* (1998).

There are different mechanisms underlying inflammatory processes. In neurogenic inflammation, some peripheral end of capsaicin sensitive sensory neurons release Substance P and other inflammatory peptide mediators. The peripheral end of these neurons also contain inhibitory opioid receptors. Opiates may be considered to be inhibitors of Substance P containing neurons and inhibitors of developing inflammation (Barnes *et al*, 1990). Opioid antagonists such as naloxone reverse such anti-inflammatory effect.

Other mechanisms of inflammatory process include the release of mediators from tissues and migrating cells, and most strongly implicated are the prostaglandins (PGs), leucotrienes (LTS), histamine, bradykinin, platelet-activating factor (PAF) and

interleukin-1 (Vane and Botting, 1990). According to these authors, these mediators are differentially involved in the evolution of the oedemas of the inflammation models.

Yesilada *et al* (1998) reported that macrophage-derived mediators, interleukin-1 (IL-1 $\alpha$  and  $\beta$ ) and tumor necrosis factor (TNF- $\alpha$ ) used in their study play a key role in inflammatory and immune responses, based on their occurrence at inflammatory sites and their ability to induce many of the hallmarks in the inflammatory response. The inhibition of these cytokines could be employed as a criterion for the evaluation of anti-inflammatory effect (Durum and Oppenheim, 1989).

In the present study, egg albumin-induced oedema model which has the capacity of detecting acute inflammation was adopted. It revealed that *S. bicolor* leaf base extract had no inhibitory effect on the oedema at the doses of 100 and 200 mg/kg i.p. The oedema was however increased significantly ( $p < 0.05$ ) at the dose of 400 mg/kg i.p. This suggests that the precursors of the inflammatory process were not attenuated by 100 and 200 mg/kg i.p. doses. The increased oedema at the dose of 400 mg/kg i.p. is indicative of enhanced inflammatory activity at this dose. It suggests an enhancement of one or more of the inflammatory mechanisms. Further experimentation will be required to elucidate the possible mechanism(s).

However, worth noting at this point is that inflammatory response is essentially a 'protective' and 'restorative' response in which the body attempts either to return to the pre-injury condition or to repair itself after inflicted injury (Ward, 1978). It is only if inflammatory response is 'aberrant' that a serious consequence may occur (Henson, 1978). The enhancement of inflammatory process at 400 mg/kg i.p. dose should

therefore be taken as an advantage. The result is in line with that of activated charcoal clearance assay for macrophage phagocytosis where only the extract dose of 400 mg/kg p.o. strongly stimulated phagocytic rate.

These two effects (phagocytosis and inflammation) usually associated with non-specific type of immunity as the first line of immune response of the host on encountering a foreign configuration may have contributed strongly to *S. bicolor* leaf base extract being used ethnomedicinally for body defence and in cancer cases.

Investigation of T-lymphocyte requires both numerical and functional assessment. The enumeration of T-lymphocytes ('T' cell count) was done on peripheral blood samples (*in vitro*) using the spontaneous sheep red cell rosette (E-rosette) assay. The result showed that the rats after 14 days treatment had decreased percentage of T-lymphocytes at all the tested doses. This decrease was significant at 200 mg/kg p.o. dose and corroborated the decreased lymphocyte counts at 100 and 200 mg/kg p.o. obtained in the haematology test after 14 days treatment with the extract. This suggests that the T-cells were possibly not sensitized. Infact, the non-significant effect of the extract on the sheep red blood cell-induced antibody titre and decrease in T-lymphocyte count of rats treated for 14 days generally suggest that both B – and T-lymphocytes may not have been sensitized/activated strongly by the extract. The implication therefore is that the impact of *S. bicolor* leaf base extract on specific immune response, involving humoral (B-lymphocytes) and cellular (T-lymphocytes) may not be as pronounced as those of non-specific immune responses involving phagocytosis and inflammation. Further experimentation is definitely required to resolve the relative contribution of *S. bicolor*

leaf base extract on humoral and cell-mediated immune responses as well as non-specific protective immunity.

Although the main thrust of this project was to ascertain the basis for the ethnomedicinal use of *S. bicolor* leaf base extract in body defence, general pharmacological screening of the extract was equally carried out. This was important for establishment of the possible effects of the extract on other body systems considering that a drug may be useful and indicated for a particular ailment yet having other desirable and undesirable effects on other body systems such as the central nervous system, gastrointestinal system, cardiovascular system, reproductive system etc.

The scientific revelation of the effect of the extract on these systems offers the opportunity of outlining the possible side effects of the extract when indicated for clinical use. It is also useful in stating the health conditions under which the extract (a potential drug) must or must not be used. Hence, the indications, contra-indications, side effects and precautions usually stated in drug information leaflets.

In addition, many reports have shown that research into ethnomedicinal uses of plants could lead to entirely new pharmacological property and to the isolation of useful compounds. The scientific proof of the ethnomedicinal use of *Catharanthus roseus* as antidiabetic was not successful. It was rather discovered that it has antileukaemic properties (Sim, 1971), Taylor and Farnsworth, 1973). Vinblastine sulphate (Velban®) or vincristine sulphate (Oncovin®) are the alkaloid derivatives available as injections of choice for leukaemia chemotherapy (Sim, 1971; Todd, 1978). *Rauwolfia serpentina* was used traditionally for mental illness, fever cure, antidote for snake bite and scorpion

stings. Scientific reports have it that the active principles have no antidote properties against snake bites or scorpion sting (Sofowora, 1982). However, reserpine with some of the other rauwolfia alkaloids have been shown to be responsible for the sedative properties of the roots. The alkaloid also exhibits antihypertensive properties and is used in modern medical practice to treat certain cases of mild hypertension as well as cases of anxiety (Sofowora, 1982).

Cotecxin® which is an antimalarial drug recommended for use by World Health Organization (WHO) in malaria endemic parts of the world contains dihydroartemisinin, which is the active and potent derivative of artemisinin. Artemisinin was long used as anti-pyretic agent. However, the failure of the commonly used anti-malarials led to research and development of more potent forms of artemisinin group as antimalarials. (Klayman, 1985; Bharati, 2006).

In the light of all these examples, subjection of *S. bicolor* leaf base extract to pharmacological evaluation was highly justifiable.

The results of the pharmacological studies carried out on the extract revealed a significant ( $P < 0.05$ ) reduction of spontaneous motor activity in mice. The spontaneous motor activity is a model that has been used in laboratory animals to evaluate the gross behavioural effects of drugs (Hsieh *et al.*, Carpenedo *et al.*, 1994; File and Fernandes, 1994). The model measures the level of excitability of the central nervous system (Masur *et al.*, 1971) which correlates well with drug effects in humans. Agents that suppress this behaviour usually do so through central inhibition (Adzu *et al.*, 2002). The significant ( $P < 0.05$ ) reduction in the spontaneous motor activity by the *S. bicolor*

leaf base extract therefore suggests a reduction in the excitability of the central nervous system which could be suggestive of sedative activity. Ozturk *et al*, (1996) reported that the decrease in the activity may be closely related to sedation resulting from CNS depression.

Similar effect was seen in the significant ( $P < 0.05$ ) reduction of the number of head dips in the hole board test by the extract. This test is a measure of exploratory behaviour (File and Wardill, 1975; Crawley, 1985) that reveals sedative activity of agents (File and Pellow 1985; Amos *et al*, 2001). The test has also been accepted as a parameter for the evaluation of anxiety conditions in animals (Crawley, 1985). The extract therefore possibly has sedative property.

Apomorphine acts directly on the post-synaptic dopamine D-2 receptors to induce hyperactivity and stereotypic behaviour. Inhibition of apomorphine-induced climbing behaviour in mice is suggestive of D1 and D2 receptor inhibition (Moore and Axton, 1988).

The ability of a drug to antagonize apomorphine-induced climbing behaviour has been correlated to central depressant activity with potential neuroleptic effect (Protais *et al.*, 1976; Costal *et al.*, 1978). The inability of *S. bicolor* leaf base extract to inhibit apomorphine-induced stereotypic behaviour possibly suggests non-inhibition of the central dopaminergic neurotransmission. It also indicates that the extract is not a potential neuroleptic.



Potentiation of pentobarbitone-induced hypnosis may be attributed to an action on the central mechanisms involved in the regulation of sleep (Chindo *et al*, 2003) or an inhibition of pentobarbital metabolism (Kaul and Kulkarni, 1978). Endogenous neurotransmitters in the brain especially dopamine and GABA are implicated in the mechanism of sleep (Osuide and Wambebe, 1980). It is generally accepted that the sedative effects of drugs can be evaluated by measurement of pentobarbital sleeping time in laboratory animals (Ming-Chin Lu, 1998; Carpenedo, 1994; Gamaniel *et al*, 1998). Prolongation of pentobarbital-induced hypnosis is suggestive of central depressant activity of a compound (Perez *et al*, 1998).

The present study showed that the crude leaf base extract of *S. bicolor* administered once prolonged pentobarbital induced-hypnosis while the aqueous and the ethylacetate fractions of the crude extract had no effect on the pentobarbital-induced hypnosis. This indicates that the extract may not have acted via dopaminergic pathways as indicated by apomorphine-induced stereotypic behaviour test but possibly by enhancing the central inhibitory effect of GABA or by inhibiting pentobarbital metabolism or via other mechanisms that may be remotely involved in the mechanism of sleep. These effects were not seen in the aqueous and ethylacetate fractions of the crude extract indicating that the pharmacological constituents of the plant responsible for this effect may have been lost or distorted due to the fractionation.

This result was further buttressed by the microsomal enzyme test result that also showed prolongation of pentobarbital-induced hypnosis after 6-days treatment with the crude leaf base extract. This observation was similar to that of cimetidine (100 mg/kg p.o.), which is a known microsomal enzyme inhibitory drug. Microsomal enzymes are

associated with a number of drug metabolisms. Administration of microsomal inhibitor reduces metabolic effect of the microsomal enzyme, producing effects of the drug with longer duration (Grant, 2001). The crude extract may have therefore inhibited the metabolic effect of microsomal enzymes on pentobarbitone prolonging its hypnotic effects.

The test for motor co-ordination (rota rod performance) was adopted to evaluate the effect of the extract on the physical performance, endurance and possible neuromuscular inhibition. The study revealed that the extract did not produce any effect on motor co-ordination. This therefore suggest that the extract has centrally-mediated actions (based on the inhibitory effects seen in other studies) and not through peripheral neuromuscular blockade (Perez, *et al*, 1998).

The use of abdominal constriction (writhing test) model was adopted for the evaluation of the crude leaf base extract for antinociceptive effect on chemical pain. The writhing response is thought to partly involve local peritoneal receptors (Bentley *et al*, 1983; Mat *et al*, 1997; Atta and Alkofahi, 1998). The result showed that the extract significantly ( $P < 0.05$ ) and dose-dependently reduced the number of acetic acid-induced abdominal constrictions (writhes) in mice. This probably suggests an antinociceptive property. This effect progressed over the 120 min. (2 h) observation period suggesting a possible long time of prolongation of antinociception. Although the use of abdominal constriction (writhing) model for detection of antinociceptive activity has been reported to be more sensitive, when compared with other models such as tail flick model (Collier *et al*, 1968; Bentley *et al* 1981), the present study revealed that antinociceptive evaluation of the extract carried on mechanical pain using tail flick test gave similar

results to that of writhing test model. The extract was able to increase the ability of mice to withstand force-induced pain (mechanical pain). The antinociceptive effect was also observed throughout the observation period of 120 min. (2 h). This further showed prolonged antinociceptive property and is an indication that the leaf base extract has the potential of being developed into analgesic.

Tail flick test for aqueous and ethylacetate fractions also revealed that the antinociceptive property was retained in the aqueous fraction while the effect was not seen in the ethylacetate fraction. This could mean that the component responsible for antinociception is polar in nature.

These results corroborate the ethnomedicinal use of the plant parts for stomachache, breast disease, tubercular swelling, kidney and urinal complaints etc. These conditions are usually associated with pain. The antinociceptive property of the plant leaf may therefore have been taken advantage of traditionally, for pain relief. This may also be a reason for the inclusion of *S. bicolor* as one out of the four herbal components (*Piper guineenses* seeds, *Pterocarpus osun* stem, *Eugenia caryophyllum* fruit and *Sorghum bicolor* leaves) of sickle cell drug (NIPRISAN<sup>®</sup>) developed by National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, considering that sickle cell condition is associated with pain.

Analgesics are classified into two – opioid analgesics (which include morphine and related compounds such as codeine) and the antipyretic analgesics (non-opioid analgesics which include aspirin, acetanilide and phenacetin).

The opioid analgesics are more consistently effective than antipyretic analgesics in pain associated with trauma or with deep structures such as viscera. The antipyretic analgesics however, are more useful in the treatment of pain associated with headache, connective tissue, arthralgia and pains arising from integumental structures rather than viscera.

Drugs such as narcotics (which opiates are) act mainly centrally while drugs such as aspirin, hydrocortisone and dexamethasone (which are non-opioids) are primarily peripherally acting (Ahmadiani *et al*, 1995). In the present investigation, formalin test was adopted to elucidate the possible site(s) (central, peripheral, or both) of antinociceptive activity observed in the extract. The result revealed that the extract dose-dependently reduced formalin-induced pain in both early (0-10 min) and late (15 – 60 min) phases of the experiment. However, the percent pain inhibition in the early phase was more than the inhibition in the late phase of the experiment. This was unlike aspirin that showed higher percent pain inhibition in the late phase. Dubuisson and Dennis (1997) and Tjolsen *et al* (1992) reported that in formalin test, nociception occurs in two phases. The first phase starts immediately after formalin injection and continues for 5 min, after which nociception appears to diminish. The second phase is marked by a return to high levels of nociception beginning 15 – 20 min. after formalin injection and continuing for 60 min. The first phase is probably a direct result of stimulation of nociceptors in the paw, while the second phase may reflect the inflammation process, and at least to some degree, the sensitization of central nociceptive neurons (Coderre *et al*, 1990; Coderre and Melzack, 1992). This method is very useful for elucidating the mechanism of pain and analgesia (Tjolsen *et al*, 1992).

Drugs such as narcotics which act mainly centrally, inhibit both phases of formalin-induced pain while drugs, such aspirin, hydrocortisone and dexamethasone which are primarily peripherally acting only inhibit the late phase (Chen *et al*, 1995; Elisabetsky *et al*, 1995; Santos *et al*, 1995). Therefore, the inhibitory action of the extract on both early and late phases suggest that the central mechanism may be involved. Also to note is that the second phase of formalin test is related to a peripheral inflammatory process. However, the earlier report on the effect of the extract on inflammation showed that the leaf base extract could not inhibit inflammation (oedema) induced by fresh egg albumin. It can therefore be deduced that the peripheral mechanism may not be involved in the antinociceptive effect of *S. bicolor* leaf base extract. This further confirms the centrally-related activities observed in the earlier reported studies. This could therefore mean that the extract may be of the opioid analgesic type rather than the antipyretic analgesic. This is also in line with the report of Carlisson and Juma (1987), that tail flick test is very sensitive to centrally acting drugs. Thus, analgesic effects of the extract in the tail flick test model provide additional evidence for central antinociceptive action of the extract. Therefore the analgesic effect of *S. bicolor* leaf base extract on tail flick test signifies central antinociceptive action. Subsequent study will involve the interaction of the antinociceptive action of the extract with opioid antagonists such as naloxone, to see if the effect could be reversed as is typical of opioid analgesics.

It is important to note that some drugs are known to be clinically effective analgesics, even antipyretics but lack significant anti-inflammatory properties e.g. phenacetin, acetaminophen while other drugs are potent anti-inflammatory agents but lack or have only weak analgesic properties e.g. phenylbutazone. Others have both analgesic and anti-inflammatory properties e.g. aspirin. The present investigation has shown that *S.*

*bicolor* leaf base extract did not inhibit egg albumin-induced oedema (inflammation), while acetic acid-induced writhing test on chemical pain and tail flick test on mechanical pain showed it to have a significant analgesic property. Although the antipyretic activity of the extract is yet to be evaluated, it is possible that the extract belongs to the same group as phenacetin, acetaminophen which have analgesic but no anti-inflammatory property.

Other evaluations carried out on *S. bicolor* leaf base extract revealed that the extract significantly ( $P < 0.05$ ) and dose-dependently reduced the propulsive movement of charcoal meal through the gastrointestinal tract. This is indicative of reduction in peristaltic activity and ultimately reduction in gastrointestinal motility. This finding has implications on the rates of gastric emptying as well as intestinal movement in general.

The inhibitory effects on gastrointestinal motility were also observed on the aqueous and ethylacetate fractions of the leaf base extract. However, the ethylacetate fraction showed higher propulsion inhibitory percentages than the aqueous fraction. This shows that the plant constituent(s) responsible for the antimotility activity were not lost after fractionation. They however seem to reside more in the ethylacetate fraction.

It was also observed that the propulsion inhibitory effects of both aqueous and ethylacetate fractions of the extract were much higher than that of the tested dose of atropine (0.1 mg/kg i.p.). A delay in gastric emptying will prevent speedy evacuation of the stomach contents (Bertaccini *et al*, 1981; Akah *et al*, 1998). This probably explains the significant ( $P < 0.05$ ) inhibition (100 %) of castor oil induced diarrhoea by the crude extract. This effect was comparable to that of loperamide (10 mg/kg i.p.). Castor oil is

classified as a stimulant laxative (Dinesh *et al*, 1999). Antimotility drugs such as loperamide block the actions of castor oil and are used to relieve diarrhoea. It is therefore possible that the crude leaf base extract inhibited castor oil-induced diarrhoea via the mechanism of gastro-intestinal motility inhibition, which is spasmolytic effect. According to the report of Di Carlo *et al* (1994), drugs affecting intestinal motility and secretion also possess antidiarrhoeal activity. It should however be noted that antimotility effect is just one out of different other antidiarrhoeal mechanisms. Further studies are required to elucidate other possible antidiarrhoeal mechanism(s) for the extract.

Test of the effect of the extract on feed and water intake of rats treated once in the morning for seven (7) days showed that amphetamine, 1.5 mg/kg p.o. (a sympathomimetic agent which is a potent CNS stimulant with appetite suppressant property) produced a significant ( $P < 0.05$ ) decrease of morning feed intake throughout the study. On the other hand, cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.) which served as the reference appetite stimulant and the crude extract produced variable effects on the morning feed intake. These effects were only significant at different days with different doses.

However, for the feed intake recorded in the afternoons, variable effects were observed and none of the treatments (amphetamine, 1.5 mg/kg; cyproheptadine  $\alpha$ -ketoglutarate, 0.3 mg/kg p.o. or the extract, 100 – 400 mg/kg) produced any significant effect.

This result shows that amphetamine obviously impaired processes involved in appetite stimulation immediately after treatment in the morning. The variable effects observed

for cyproheptadine  $\alpha$ -ketoglutarate and the extract on morning feed intake also signify variable effects on the appetite stimulating mechanisms.

It is worth noting at this point however that lack of effect of any of the treatments (amphetamine, cyproheptadine  $\alpha$ -ketoglutarate or the extract) on the afternoon feed intake could be attributed to the biotransformation of the drugs. Metabolism, storage and excretion are reported to be the three mechanisms whereby drugs are ultimately removed from their sites of action (Grant, 2001). This therefore means that all the drugs must have been metabolized and excreted before they could exert any effect on the afternoon feed intake.

The water intake was also reduced significantly ( $P < 0.05$ ) by amphetamine after 7-days treatment. This possibly relates also with its appetite suppression effect. Cyproheptadine  $\alpha$ -ketoglutarate and the extract also produced variable effects on water. These observed effects were significant at different doses on different days. This again suggests variable effect on appetite including thirst-stimulating mechanisms.

*S. bicolor* leaf base extract produced a concentration-dependent relaxation of rabbit jejunum in contrast to concentration-dependent contractility caused by acetylcholine and histamine on the same tissues. The extract did not produce any effect on smooth muscles of guinea pig ileum except in one out of four preparations studied in which a slight relaxation was observed at extract concentrations of 1.28 – 5.12 mg/ml. These same tissues were contracted by histamine and acetylcholine.



Although the mechanism for the observed relaxation is not clear from this study, the relaxation could be the cause for the antimotility effect and subsequent anti-diarrhoeal activity observed earlier in the study. Dinesh *et al*, (1999) reported that advantage can be taken of agents that reduce intestinal motility, gastric secretory effect and are anti-spasmodic as adjunctive treatment in non-ulcer dyspepsia, irritable bowel syndrome and diverticular disease. Antispasmodics are of value for treating abdominal cramps associated with diarrhoea while antimotility drugs relieve diarrhoea. This therefore suggests that the extract has the potential of being developed into antispasmodic and/or antimotility agent. This corroborated with the use of (though the seed) of *S. bicolor* plant in folklore medicine as a remedy for diarrhoea. The plant constituent(s) responsible for the anti-diarrhoeal activity of the plant might be present in both the seed and the leaf.

Conversely, the extract contracted the smooth muscles of rat stomach fundus strip in a manner that was not concentration-dependent as did acetylcholine, histamine and 5-hydroxytryptamine. The mechanism for this contractile effect on stomach fundus requires further elucidation. This will help to explain the reason for having contractile effect in the stomach and relaxation effect in the intestine (as was demonstrated on rabbit jejunum, guinea pig ileum and mice intestinal motility).

*S. bicolor* leaf base extract did not alter the intrinsic myogenic contraction of isolated rat atria. The contractile amplitude of these tissues were increased by adrenaline.

The atrium houses the sino atrial node (SAN) from where impulse and rhythm are generated to the remaining part of the heart. The heart is influenced by autonomic

nervous system to modulate spontaneous cardiac rhythmic activity (Broadly, 1982; Willfert, 1986). The catecholamines released from the sympathetic nervous system act on post-synaptic adrenoceptors to increase heart rate and contractile force. These adrenoceptors are predominantly of the  $\beta$ -type (Carlson *et al*, 1977; Brodde, *et al*, 1982). On the other hand,  $\beta$ -adrenergic antagonists slow the heart rate and decrease myocardial contractility (Mimran and Ducailar, 1988) and these antagonists have significant effect on cardiac rhythm and automacity. The present study therefore showed that the tested concentrations of the crude leaf base extract neither produced effect similar to those of catecholamines nor those of  $\beta$ -adrenergic antagonists. This suggests that the extract may not have interfered with the mechanism(s) involved in modulation of cardiac rhythmic activity.

*S. bicolor* extract also did not alter the intrinsic myogenic contraction of rat portal vein. The spontaneous rhythmic myogenic contractions of the portal vein is also modulated by the autonomic nervous system. It also depends on the influx of extracellular calcium (Omogbai and Smith, 1990). Potassium chloride (KCl) evoked a sustained contraction of the rat portal vein which was blocked by chlorpropamide, which is thought to act by binding to and blocking an ATP-sensitive  $K^+$  channel, which has been cloned (Philipson and Steiner, 1995). The production of no alterations in the contractility of the isolated rat portal vein by the tested concentrations of the crude extract possibly implies that the extract did not interfere with any of the contractile mechanisms.

The rhythmic contractions of stilboestrol pre-treated uteri were not altered by the leaf base extract. This was validated by the contractile effects of oxytocin and acetylcholine on the same tissues. The extract therefore seems to be non-uterogenic.

In addition to this, the contractile effect of oxytocin on the tissues were not blocked by the extract. Oxytocin is known to stimulate both the frequency and force of uterine contractions. These effects are highly dependent on oestrogen (Parker and Schimmer, 2001). This justified the pretreatment of the uteri with stilboestrol, an active synthetic oestrogen (Livingstone, 1987).

Oxytocin acts via specific G protein-coupled membrane receptors most closely related to the V<sub>1a</sub> and V<sub>2</sub> vasopressin receptors (Parker and Schimmer, 2001). The inability of the extract to inhibit the contractile effect of oxytocin on rat uteri suggests that the oxytocin receptors were not blocked by the extract. The earlier ethnomedicinal report that *S. bicolor* plant is used as antiabortive agent may probably involve other mechanisms.

*S. bicolor* leaf base extract on the other hand caused minimal contraction on rat vas deferens. Acetylcholine also contracted these tissues. However, the contractile effect of acetylcholine on the tissue was blocked by atropine confirming involvement of cholinergic mechanism while the contractions produced by the extract on the tissues were not blocked by atropine. This suggests that the extract may have contracted vas deferens via mechanism(s) other than cholinergic mechanism. Mepyramine, H<sub>1</sub>-receptor blocking drug did not block the contractile effects of acetylcholine and the extract on vas deferens. This suggest that the extract did not contract the tissue via histaminergic pathway.

The present investigation has shown the immuno-pharmacological potentials of *S. bicolor* leaf base extract. It also revealed the toxicity profile of the extract in order to

determine the extent of its safety if in use as drug. This is because as important as it is for drugs to be efficacious, cheap and available, there is extreme need for drugs to be safe for short and long term uses. The fact that different substances have different toxicity levels is shown in the classification of substances into very toxic, toxic, less toxic or only slightly toxic (Lorke, 1983). This further indicates that evaluation of safety profile of a drug is paramount in the development of drugs and in their subsequent clinical uses.

Acute toxicity study was used to establish the median lethal dose ( $LD_{50}$ ) of mice and rats treated orally and intraperitoneally. The median lethal dose has been shown not to be an absolute value but an inherently variable biological parameter that cannot be compared to constants such as molecular weight or melting point (Oliver, 1986). It was therefore further reported that 'accuracy' should not be used to describe  $LD_{50}$  but 'precision'.

The precision being only relevant to the experiment for which the median lethal dose ( $LD_{50}$ ) was derived and does not increase the probability that in subsequent experiments the  $LD_{50}$  will be identical or even similar (Oliver, 1986).

It is worth noting at this point that acute toxicity ( $LD_{50}$ ) test has its limitations. These were seen in the criticisms of the use of acute toxicity ( $LD_{50}$ ) test as a parameter for assessing toxicity (Lorke, 1983; Klaasen, 2001; Timbrel, 2002). This was buttressed by the report of Orisakwe *et al* (2002) that  $LD_{50}$  does not necessarily guarantee the safety of the tested agent notwithstanding its value. According to the report, rinbacin with a high  $LD_{50}$  value induced toxic effects to the rat testis. Despite these limitations

however, acute toxicity study furnishes some useful information. For instance, it helps in the selection of dose ranges that could be used for subsequent studies. The possible clinical signs induced by the substance of investigation could manifest at this level of study. It is also applied in the establishment of therapeutic index (i.e. LD<sub>50</sub>/ED<sub>50</sub>) of drugs and xenobiotics (Rang *et al*, 2001).

The present study showed that *S. bicolor* leaf base extract caused no overt toxicity sign or death in mice and rats 72 h post oral treatment. The oral median lethal dose (LD<sub>50</sub>) of the extract was estimated to be  $\geq 2,000$  mg/kg in both mice and rats. The Organization for Economic Cooperation and Development (OECD, Paris, France) recommended chemical labeling and classification of acute systemic toxicity based on oral LD<sub>50</sub> values as: very toxic,  $\leq 5$  mg/kg; toxic,  $> 5 \leq 50$  mg/kg; harmful,  $> 50 \leq 500$  mg/kg and no label,  $>500 \leq 2,000$  mg/kg (Walum, 1998). Based on this classification, the oral LD<sub>50</sub> up to 2,000 mg/kg established for both mice and rats indicate relative oral safety. Lack of overt toxicity signs in these experimental animals also points to that fact.

On the other hand, intraperitoneal administration of the extract in mice and rats elicited toxicity signs which included calmness, dullness, increased respiration and death at doses between 1,500 – 2,000 mg/kg i.p. The intraperitoneal LD<sub>50</sub> value ranges of 1,248.0 - 1,341.6 mg/kg i.p. were estimated for the mice while that of the rat was 1,414.2 mg/kg i.p.

The study also showed that intraperitoneal administration of the ethylacetate and aqueous fractions of the extract elicited dullness, immobility and increased respiration within 10 – 12 min post administration with doses between 1,000 – 2,000 mg/kg i.p.

All the animals soon recovered from these toxicity signs and no mortality was recorded. Intraperitoneal LD<sub>50</sub> ≥ 2000 mg/kg was estimated for both the ethylacetate and the aqueous extract.

This also means that intraperitoneal administration of the extract, its ethylacetate and aqueous fraction is also relatively safe since Lorke (1983) considered LD<sub>50</sub> values > 1000 mg as safe. However, attention should be given to the calmness, dullness and increased respiration observed (though at high doses) few minutes post treatment.

The clinical signs and mortalities observed at certain dose ranges have proven that acute toxicity study (LD<sub>50</sub>) is useful after all. However, such acute toxicity data are of limited clinical application since cumulative toxic effects do occur even at very low doses. Hence, sub-acute and chronic toxicity studies are almost always invaluable in evaluating the safety profile of phytomedicines (Aniagu *et al*, 2005). This is probably the basis for suggestion that sub-chronic toxicity data be used to predict the hazard of long term, low-dose exposure to a particular compound (McNamara, 1976). In the present investigation, sub-acute toxicity study was carried out for evaluation of long term, low dose effect of *S. bicolor* leaf base extract.

The study revealed that no adverse clinical signs or toxicity sign or death was observed throughout the treatment duration of 28 days. This is in line with the acute toxicity studies where experimental animals treated orally with the extract doses < 2000 mg/kg showed neither toxicity sign nor death. This may be an indication that long term oral administration of the extract within these low dose ranges is safe.

A general increase in body weight was observed in all the groups throughout the study duration. The body weight increases for the extract-treated groups were however not significantly different from the control. According to Klaassen (2001), when animals lose appetite (anorexia), weight loss is bound to ensue due to disturbances in carbohydrate, protein or fat metabolism. The general increase in body weight observed shows that the extract possibly did not induce anorexia, an effect that could have resulted in loss of body weight. The non-significant effect of the extract on the body weight and feed intake further confirm that *S. bicolor* leaf base extract did not interfere with the nutritional benefits (e.g. weight gain, stability of appetite) expected of animals that were supplied with feed and water *ad libitum* as was suggested by Aniagu *et al.*, (2005).

Simmons *et al* (1995) reported that increased organ weight (either absolute or relative) has been observed as a sensitive indicator of organ toxicity by known toxicants. This is in line with the study and report of Wolfgang *et al* (1995) on Beagle dogs where a novel lipid was shown to induce hepatocellular hypertrophy with alanine transaminase (ALT) and aspartate transaminase (AST) being elevated, while there was a 2 – 3 fold increase in cytochrome P450 content of hepatic microsomes. This report was however disputed by the work and report of Dioka *et al* (2002) on rinbacin (a herbal preparation), that there was no increase in either the absolute or relative weight of rat organ (liver) even with increase in the biochemical parameters, which are indications of hepatotoxicity. Another report by Dalbey and Feuston (1996) on the other hand, observed an increase in liver weight of rats exposed to diisopropyl ether without any increase in biological parameters.

The present study showed that *S. bicolor* leaf base extract caused no significant changes in the relative weight of the liver, heart, spleen and lungs. 200 mg/kg p.o. dose of the extract however significantly ( $P < 0.05$ ) reduced the relative weight of the kidneys while 400 mg/kg dose caused a significant increase in the relative weight of the testes.

These results could mean that the integrity of all the above organs (with exception of the kidneys and testes) were not tampered with by the extract. However, this deduction can only be possibly true if the results of the effects of the extract on relative organ weight, serum biochemical indices and histopathology of these organs are considered together.

Gross pathological observation of the organs showed no gross abnormalities in the morphologies/features, consistencies and appearances of the liver, kidney, heart, spleen, lungs and testes of the male rats treated for 28 days with the extract. Histopathological examinations also revealed that there were no abnormalities in the kidneys, heart, spleen, liver (at 100mg/kg) and lungs of the rats. The observation of some pathological changes in the liver and testes highlighted the limitations of gross pathological examinations, which showed no abnormalities in these organs. However, it is intended to have this study repeated with special emphasis on the effect of the extract on the liver and testes considering that some of the seemingly observed pathological changes were also observed in the control slides.

Liver cell damage is characterized by a rise in plasma enzymes (aspartate aminotransferase - AST, alanine aminotransferase – ALT, etc; Aniagu *et al*, 2005). Serum biochemical analyses carried out to evaluate the effect of the extract on hepatic function indices revealed that the extract caused no significant effect on direct bilirubin,



total bilirubin, albumin, glutamate oxaloacetate transaminase (GOT) also known as aspartate aminotransferase (AST), glutamate pyruvate transaminase (GPT) also referred to as alanine aminotransferase (ALT), alkaline phosphatase and total protein.

The absence of significant changes in the relative weight of the liver, the absence of abnormalities in the morphology/features, consistency and appearance of the liver observed grossly and the non significant difference observed in the hepatic function indices suggest absence of hepatotoxicity. Some seemingly histopathological changes which were also found in the control slides have to be confirmed.

The result of the effect of the extract on renal function indices showed that the extract produced no significant effect on both urea and creatinine. It however reduced uric acid significantly ( $P < 0.05$ ) at doses of 100 and 200 mg/kg p.o. This is a reflection of the preserved renal integrity of the treated rats (Kaneko, 1989). This result also points out that therapeutic advantage can be taken of the extract's ability to reduce uric acid in hyperuricemia, a condition that can pre-dispose to renal disease, gouty arthritis, intense inflammation of soft tissues on which urate crystals are deposited (Rock *et al*, 1986).

Absence of abnormalities in the gross and histopathological examination of the kidney in addition to the observed effects on renal function indices further suggest that the excretory capacity of the kidney was not impaired.

Effect of the extract tested on serum lipid profile showed that the extract had no significant effect on cholesterol. It however reduced triglyceride significantly. In human nutrition triglycerides are the most prevalent glycerol esters encountered. They

constitute 95 % of tissue storage fat and are the predominant form of glycerol esters found in plasma. Following absorption, triglycerides are resynthesized in the epithelial cells and combine with cholesterol and a number of apolipoproteins to form chylomicrons which travel through the lymphatic system to the thoracic duct and eventually to the jugular vein (Stein, 1986). The significant reduction of triglyceride by the extract is indicative of the lipid-lowering potential of the extract in mixed hyperlipidaemic states (Aniagu *et al*, 2005). The report of Stein (1986) about the combination of triglyceride with cholesterol for eventual movement to the thoracic duct and jugular vein is indicative of clinical importance of this extract-lipid effect. This is because of the correlation existing between the serum cholesterol levels and the incidence of ischaemic and coronary heart diseases such as atherosclerosis (Stamler, 1986; Dixit *et al*, 1992).

## Chapter 6 SUMMARY, CONCLUSIONS AND RECOMMENDATION

### 6.1 Summary

A wide folkloric use of different parts of *S. bicolor* (Linn.) Pers. (Family: Gramineae; Poaceae) plant in the treatment of different ailments have been reported. It was however, the ethnomedicinal use of the leaves of the plant for the stimulation of blood production and for body defense that prompted the present investigation of the plant leaf base in the light of immuno-pharmacology. This was in consideration that a number of clinical conditions like cancer, surgery, certain drugs, HIV/AIDS and various stressors affect different components of the immune system thereby allowing opportunistic pathogens to overwhelm the host leading to secondary infections and mortality in such individuals. Also, the general pharmacological screening was undertaken to determine the extent of the desirable and/or undesirable effects of the extract on other body systems such as the central nervous system, gastrointestinal system, cardiovascular system and reproductive system. The toxicity profile of the extract was also evaluated to determine the extent of its safety for short and long term uses as drug.

The base of the leaves attached to the suckers of *S. bicolor* plant were successively cold macerated with 70 % v/v methanol over 96 h periods producing yields of 23.6 % w/w extract. The crude extract was further partitioned into non-polar, medium polar and very polar fractions using hexane, ethylacetate and water producing yields of 0.5 %, 95.9 % and 3.6 % w/w respectively.

The effects of *S. bicolor* crude leaf extract were tested on haematological indices (Hb, PCV, total WBC, DLC, total RBC, platelet count, bleeding time and clotting time) and immunomodulatory models which included delayed type hypersensitivity reaction, humoral response in normal and cyclophosphamide-induced immunodeficiency,

activated charcoal clearance assay for macrophage phagocytosis, anti-inflammatory effect, 'E' rosettes for estimation of T-lymphocytes.

Also carried out were some pharmacological studies related to central and peripheral nervous systems. These included studies on spontaneous motor activity, exploratory behaviour, apomorphine-induced stereotypic behaviour, pentobarbitone-induced sleep, test for motor co-ordination (rota-rod performance), acetic acid-induced writhing test (test on chemical pain), tail flick test (test on mechanical pain) and formalin test. Studies related to gastrointestinal system included intestinal transit test, castor oil-induced diarrhoeal test, effects on isolated rabbit jejunum, guinea pig ileum and rat stomach fundus strip. Cardiovascular system related studies included tests on isolated rat atria and rat portal vein while those related to the reproductive system included effects on rat uterus and rat vas deferens. The toxicity profile of the extract was also evaluated on some experimental animals using both acute and sub-acute toxicity models. The partitioned fractions were tested for effects on intestinal propulsion, tail flick test and pentobarbitone-induced sleep as bioassay guided models.

The results revealed that 70 % methanolic extract of *S. bicolor* (100 – 400 mg/kg p.o.) produced no significant change in the PCV, Hb, total WBC, total RBC and bleeding time. The dose of 100 mg/kg p.o. produced a significant ( $p < 0.05$ ) increase in neutrophil and decrease in lymphocyte. No significant effects were seen in the monocyte, basophil, eosinophil and clotting time. Platelet count was significantly ( $p < 0.05$ ) increased at the dose of 400 mg/kg p.o. All the groups (including the control) showed enhanced delayed type hypersensitivity reaction. However, the only significant ( $P < 0.05$ ) increase was with 400 mg/kg p.o. dose at the 48 h. No significant difference occurred in the sheep red

blood cell-induced antibody titre of the extract-treated mice. Studies on activated charcoal assay showed that the extract only exhibited strong stimulation of phagocytic rate at the dose of 400 mg/kg p.o. The extract (100 – 400 mg/kg i.p.) did not inhibit fresh egg albumin-induced inflammation in rats. Significant ( $p < 0.05$ ) enhancement in the induced oedema was rather recorded at 400 mg/kg i.p. dose. The leaf extract of *S. bicolor* produced a decrease in the number of rosetted cells. This decrease was significant at the dose of 200 mg/kg p.o.

The extract (100 – 200 mg/kg i.p.) produced a significant ( $p < 0.05$ ) reduction of spontaneous motor activity of mice. The study also revealed a significant ( $p < 0.05$ ) reduction in the exploratory activity of mice by the leaf extract (100 – 400 mg/kg i.p.). The reduction was however not as great as that of diazepam (1 mg/kg i.p.). *S. bicolor* leaf extract (100 – 400 mg/kg i.p.) did not inhibit apomorphine-induced stereotypic behaviour of mice. The leaf extract of *S. bicolor* caused a reduction in onset of pentobarbitone-induced sleep. This effect was not dose-dependent and was only significant ( $p < 0.05$ ) at the dose of 100 mg/kg i.p. The extract however prolonged the duration of pentobarbitone-induced sleep in a non dose-dependent manner. The prolongation was only significant at the dose of 100 mg/kg i.p. Diazepam (1 mg/kg i.p.) on the other hand significantly ( $p < 0.05$ ) caused a reduction in the sleep onset and prolongation of the sleep duration. The aqueous (100 – 400 mg/kg i.p.) and the ethylacetate (100 – 400 mg/kg i.p.) fractions of *S. bicolor* leaf base extract produced no significant effect on both the onset and duration of pentobarbitone-induced sleep in mice while diazepam (1 mg/kg i.p.) caused a significant ( $p < 0.05$ ) prolongation of the sleep duration. Cumulative 6-days administration of the leaf extract (100 – 400 mg/kg p.o.) produced no significant effect on both the onset and duration of sleep of mice treated with

pentobarbitone sodium (30 mg/kg i.p.). Phenobarbitone (1 mg/kg p.o.) produced no significant reduction of sleep onset but a significant ( $p < 0.05$ ) reduction in the duration of sleep while cimetidine (100 mg/kg p.o.) produced a significant reduction of sleep onset and significant ( $p < 0.05$ ) prolongation of duration of pentobarbitone-induced sleep in mice. On the other hand, the cumulative 6-days administration of the extract (100 – 400 mg/kg p.o.) caused no significant effect on the sleep onset of rat treated with pentobarbitone (40 mg/kg i.p.). The pentobarbitone sleep duration was however prolonged in these rats by all the doses of the extract. These effects were not dose-dependent and was only significant at dose of 400 mg/kg p.o. Phenobarbitone (1 mg/kg p.o.) produced some reduction in onset and duration of pentobarbitone-induced sleep while cimetidine (100 mg/kg p.o.) produced a significant ( $p < 0.05$ ) prolongation of the sleep.

The leaf extract (100 – 400 mg/kg i.p.) did not produce any significant effect on the rota-rod performance (motor coordination) of mice. Acetic acid-induced writhing test revealed that the leaf extract (100 – 400 mg/kg i.p.) significantly ( $p < 0.05$ ) reduced the number of acetic acid-induced abdominal constrictions in mice. The anti-nociceptive effect was dose-dependent and occurred throughout the observation period of 120 min. The result was comparable to that of aspirin (100 mg/kg i.p.). Tail flick test also showed that the leaf extract (100 – 400 mg/kg i.p.) increased threshold to mechanical pain in mice. The effect was not dose-dependent but occurred at all the observation intervals of 30 min over 120 min period. However, they were only significant at 200 and 400 mg/kg i.p. doses. The results compared favourably with ASA (100 mg/kg i.p.). The extract (100 – 400 mg/kg i.p.) dose-dependently reduced formalin-induced pain in both early (0 – 10 min) and late (15 – 60 min) phases of the test. However, the percent pain inhibition

was much higher in the early phase than the late phase. This was unlike ASA (150 mg/kg i.p.) that showed higher percent pain inhibition in the late phase.

*S. bicolor* leaf extract (100 – 400 mg/kg i.p.) significantly ( $p < 0.05$ ) decreased the propulsive movement of charcoal meal through the gastrointestinal tract. The effect was dose-dependent. Carbachol (1 mg/kg i.p.) on the other hand significantly ( $P < 0.05$ ) increased the intestinal propulsion. Also, the aqueous fraction of the leaf extract (100 – 400 mg/kg i.p.) reduced the charcoal meal movement. The inhibitory effect was dose-dependent and was only significant ( $p < 0.05$ ) at 100 mg/kg i.p. dose. Ethylacetate fraction of the extract (100 – 400 mg/kg i.p.) also reduced charcoal meal movement at all the tested doses. The effect was not dose dependent but was significant ( $p < 0.05$ ) at all the tested doses. The dose of 400 mg/kg i.p. produced the highest inhibitory effect. The propulsive inhibitory effects of the aqueous and ethylacetate fractions of *S. bicolor* leaf extract (100 – 400 mg/kg i.p.) were more pronounced than that of atropine (0.1 mg/kg i.p.). The leaf extract (200 – 400 mg/kg i.p.) exhibited marked anti-diarrhoeal activity with 100 % inhibition. This effect compared favourably with that of loperamide (10 mg/kg i.p.). Amphetamine (1.5 mg/kg p.o.) produced a significant ( $P < 0.05$ ) decrease in morning feed-intake through 7-days treatment. The extract (100 – 400 mg/kg p.o.) however produced variable effects on the feed intake as did cyproheptadine  $\alpha$ -ketoglutarate (0.3 mg/kg p.o.).

*S. bicolor* leaf extract (0.04 – 2.56 mg/ml) produced a concentration-dependent relaxation of rabbit jejunum. This was in contrast to the contractility caused by acetylcholine (0.004 – 0.016  $\mu$ g/ml) and histamine (0.4 – 0.8  $\mu$ g/ml) on the same tissues. The extract (0.04 – 5.12 mg/ml) produced no effect on smooth muscles of

guinea pig ileum except in one out of four preparations studied where slight relaxation was observed at concentrations of 1.28 – 5.12 mg/ml. Histamine (0.04 – 0.16 µg/ml) and acetylcholine (0.02 – 0.16 µg/ml) contracted the same tissues. *S. bicolor* leaf extract (0.04 – 5.12 mg/ml) contracted smooth muscles of rat stomach fundus strip. Acetylcholine (0.04 – 0.16 µg/ml), histamine (0.08 – 0.16 µg/ml) and 5-hydroxytryptamine (5-HT; 0.004 – 0.016 µg/ml) also contracted these tissues in a concentration-dependent manner.

The leaf extract (0.4 – 3.2 mg/ml) produced no effect on rat atria whereas adrenaline (0.04 µg/ml) contracted these tissues. *S. bicolor* leaf extract (0.04 – 5.12 mg/ml) did not alter the intrinsic myogenic contraction of rat portal vein. Adrenaline (0.04 – 0.16 µg/ml) however increased the contractility of these tissues.

The leaf extract (0.04 – 5.12 mg/ml) produced no effect on the non-pregnant rat uterus. Oxytocin ((0.004 – 0.016 µg/ml) and acetylcholine (0.02 – 0.08 µg/ml) contracted this tissue in a concentration-dependent manner. The contractile effects of oxytocin ((0.008 – 0.016 µg/ml) were not blocked by the extract (0.64 – 2.56 mg/ml). The study showed that the leaf extract (0.4 – 25.6 mg/ml) caused minimal contractions of rat vas deferens. These contractions were not concentration-dependent. Acetylcholine (0.04 – 0.16 µg/ml) produced concentration-dependent contraction of the tissue. Atropine (0.4 µg/ml) blocked the contractile effect of acetylcholine (0.16 µg/ml) on the tissue but could not block the contractile effect of the extract (3.2 mg/ml) on the same tissue. The contractile effects of acetylcholine (0.16 µg/ml) and *S. bicolor* leaf extract (3.2 mg/ml) were not blocked by mepyramine (0.16 µg/ml).



The estimated oral median lethal dose (LD<sub>50</sub>) of the extract in both mice and rats was  $\geq$  2,000 mg/kg p.o. The estimation of the intraperitoneal median lethal dose (LD<sub>50</sub> i.p.) in rats based on 24 h post treatment assessment was  $\geq$  2,000 mg/kg i.p. while that based on the 48 h post treatment assessment was calculated to be 1,414.2 mg/kg i.p. The estimated intraperitoneal median lethal dose in mice was 1,248.0 mg/kg i.p. and 1,341.6 mg/kg i.p. for 24 h and 48 h post treatment observations respectively. The estimated intraperitoneal LD<sub>50</sub> for both the ethylacetate and aqueous fractions of *S. bicolor* leaf was  $\geq$  2000 mg/kg i.p. in mice. The various LD<sub>50</sub> values translated to relative safety of *S. bicolor* leaf.

Subacute toxicity study (28 days treatment) revealed that the extract (100 – 400 mg/kg p.o.) did not cause any adverse clinical sign, toxicity sign or mortality. It did not produce significant change in feed intake and body weight. Water was variably consumed with only significant (P<0.05) increase recorded in week 2 for 100 mg/kg p.o. dose. No significant change occurred in the relative organ weight except at 200 mg/kg dose that produced a significant (P<0.05) reduction in the relative weight of kidneys and 400 mg/kg dose that caused a significant (P<0.05) increase in the relative weight of testes. No gross abnormality of the organs was detected. Histopathological examinations revealed that there were no abnormalities in the kidneys, heart, spleen and lungs. No abnormality was also seen in liver at 100 mg/kg p.o. dose. However, some histopathological changes such as nuclear fragmentation, a few collapsed blood vessels and some cellular infiltrations were seen in the liver at the dose of 200 mg/kg p.o. while the testes showed some seemingly fibrotic tissues at 400 mg/kg p.o. dose. These features were also seen in some of the control slides suggesting that they may not really be abnormalities due to the extract. The study also revealed no significant effect on the

haematological indices and hepatic function indices. Study of effect on renal function indices showed no significant effect on urea and creatinine while doses of 100 and 200 mg/kg p.o. significantly ( $P < 0.05$ ) reduced uric acid. Study of effect on serum lipid profile also showed no significant effect on cholesterol while triglyceride was significantly reduced at 200 mg/kg p.o.

## 6.2 Conclusion

The non significant effect of *S. bicolor* leaf base extract on most of the haematological indices possibly suggests the inability of the extract to activate the haemopoietic sites (such as bone marrow, spleen and liver) where blood cell production occurs. This result did not therefore corroborate the folkloric use of the plant leaves for blood stimulation.

The study also revealed that the effect of *S. bicolor* leaf base extract on specific immune response involving humoral (B-lymphocytes) and cellular (T-lymphocytes) may not be as pronounced as those of non-specific immune responses involving phagocytosis and inflammation. These two effects (phagocytosis and inflammation) usually associated with non-specific type of immunity as the first line of immune response of the host on encountering a foreign configuration may have contributed strongly to the ethnomedicinal use of the plant leaves in disease prevention and cancer cases.

The pharmacological models adopted for the evaluation of the effect of *S. bicolor* leaf extract on central and peripheral nervous systems revealed that the extract possibly has sedative and anti-nociceptive properties. The extract exhibited centrally-mediated actions and not peripheral neuromuscular blockade. These results are indicative that *S. bicolor*

leaf extract has a potential of being developed as a remedy for pain and some of the central nervous system related problems.

The present study also showed significant ( $p < 0.05$ ) inhibitory effect of *S. bicolor* leaf extract on some of the gastrointestinal system-related activities. This therefore suggests that it also has the potential of being developed into antispasmodic and/or antimotility agent. This corroborated the use of (though the seed) of *S. bicolor* plant in folklore medicine as a remedy for diarrhoea.

*S. bicolor* leaf base extract neither altered the intrinsic myogenic contraction of isolated rat atria nor those of isolated rat portal vein. The absence of contractile alterations observed on the rat atria and portal veins could possibly be extended to mean that the extract may not have detrimental effect on the cardiovascular system if in use as drug.

The lack of alteration in the rhythmic contractions of rat uteri by *S. bicolor* leaf extract suggests that the extract could be non-uterogenic and may therefore be a contributory reason why it may not be contra-indicated in pregnancy if developed as drug. The extract on the other hand caused minimal contractions on the isolated rat vas deferens. This could have effect on the secretory activity.

Acute toxicity evaluation carried out showed relative safety for both orally and intraperitoneally administered *S. bicolor* leaf base extract. The sub-acute toxicity evaluation also showed that a relatively long term administration of *S. bicolor* leaf base extract may not have detrimental effect on the body weight, hepatic function, renal function as well as on the lipid profile (which could lead to some cardiovascular-related

disease conditions such as atherosclerosis). This also showed relative safety and possibly explains the wide acceptability in folklore medicine.

### **6.3 Recommendations**

It was observed in the study that haematological results did not corroborate the ethnomedicinal use of the plant leaves for stimulation of blood production. A repeat of haematological studies using the entire leaves of the plant instead of just the dark red base of the leaves attached to the suckers of the plant is therefore recommended.

The antinociceptive studies carried out on *S. bicolor* leaf base extract suggested that the extract could have acted via central mechanism as do opioid analgesics. The interaction of the antinociceptive action of the extract with opioid antagonists such as naloxone (to see if the effect could be reversed as is typical of opioid analgesics) is therefore recommended.

The present study revealed a dose-dependent relaxation of rabbit jejunum and slight relaxation of one out of four isolated guinea pig ileum preparations. These observations were suggested to have contributed to the antimotility effect and anti-diarrhoeal activity of the extract. The elucidation of the detailed mechanism(s) for the observed relaxation of the tissues are highly recommended.

The present evaluation was limited to the crude extract and the fractions of the extract. This was due to lack of adequate facilities for the identification of the active principle(s)

responsible for the observed activities. It is therefore recommended that identification of these active components be pursued as at when the necessary facilities are available on ground or following interlaboratory visits.

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**Appendix I:** Activated charcoal clearance assay for macrophage phagocytosis of rats treated with methanolic extract of *S. bicolor* leaf base (100, 200 and 400 mg/kg p.o.)

<i>Treatment</i>	<i>Time (min)</i>			
	<i>5</i>	<i>10</i>	<i>15</i>	<i>30</i>
Control	0.053 ± 0.020	0.045 ± 0.020	0.059 ± 0.020	0.045 ± 0.020
<i>S. bicolor</i>				
(mg/kg p.o.)				
100	0.085 ± 0.010	0.090 ± 0.003	0.077 ± 0.010	0.096 ± 0.004
200	0.092 ± 0.002	0.092 ± 0.010	0.092 ± 0.003	0.098 ± 0.010
400	0.102 ± 0.010	0.097 ± 0.003	0.099 ± 0.010	0.092 ± 0.004

Note: Values are mean absorbance ± SEM

**Appendix II:** Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.)  
on fresh egg albumin-induced paw oedema in rats

<i>Treatment</i>	<i>Time (min)</i>						
	0	20	40	60	80	100	120
Control	0.87	1.23	1.25	1.55	1.83	1.77	1.64
		[0.36]	[0.38]	[0.68]	[0.96]	[0.90]	[0.77]
<i>S. bicolor</i>							
100 mg/kg i.p.	1.00	1.59	1.59	1.70	1.80	1.91	1.85
		[0.59]	[0.59]	[0.70]	[0.80]	[0.91]	[0.85]
200 mg/kg i.p.	0.90	1.37	1.35	1.50	1.59	1.79	1.82
		[0.47]	[0.45]	[0.60]	[0.69]	[0.89]	[0.92]
400 mg/kg i.p.	0.94	1.33	1.63	1.58	1.99	2.01	2.09
		[0.39]	[0.69]*	[0.64]	[1.05]	[1.07]	[1.15]*
Aspirin	0.79	1.09	1.15	1.32	1.52	1.51	1.53
(100 mg/kg i.p.)		[0.30]	[0.36]	[0.53]	[0.73]	[0.72]	[0.74]

[ ] = Average oedema in cm<sup>3</sup>

\* = p < 0.05; significant increase in oedema (enhanced inflammatory activity; ANOVA, Student t-test)

**Appendix III:** Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on rat lymphocyte-sheep red blood cell rosetting

<i>Treatment</i>	<i>Mean rosetted cells</i>	<i>Mean non-rosetted cells</i>	<i>Rosetted cell percent</i>
Control	14.6 ± 1.3	23.8 ± 2.7	38.0
<i>S. bicolor</i>			
100 mg/kg p.o.	10.8 ± 3.5	21.8 ± 3.9	33.1
200 mg/kg p.o.	7.8 ± 2.4*	30.8 ± 3.3	20.2
400 mg/kg p.o.	20.8 ± 3.4	35.0 ± 5.5	37.3

\*= p<0.05; statistical difference between treated and control group (one-way ANOVA; Student t-test).

**Appendix IV:** Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on spontaneous motor activity in mice

<i>Time (min.)</i>	<i>Mean Activity Count ± SEM</i>			
	<i>Control</i>	<i>Ext. 100 mg/kg</i>	<i>Ext. 200 mg/kg</i>	<i>Ext. 400 mg/kg</i>
0	465.0 ± 121.8	618.0 ± 46.4	674.8 ± 44.6	512.5 ± 97.0
30	581.0 ± 53.7	240.0 ± 46.0*	111.2 ± 29.4*	312.5 ± 98.5*
60	247.5 ± 45.3	53.2 ± 25.8*	52.0 ± 19.1*	38.3 ± 24.4*
90	133.6 ± 21.9	120.2 ± 36.5	43.0 ± 11.5*	38.2 ± 17.1*
120	178.6 ± 20.6	279.2 ± 174.5	69.2 ± 27.0*	114.3 ± 72.5

Note: Values are expressed as mean ± SEM (n=6).

\* = p<0.05; statistical difference between treated and control groups (ANOVA; Student t-test).



**Appendix V:** Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on glacial acetic acid-induced abdominal constriction in mice

<i>Treatment</i>	<i>Time (min)</i>	<i>Mean writhe ± SEM</i>	<i>% Abdominal constriction in relation to control</i>
Control	30	71.0 ± 4.5	100.00
<i>S. bicolor</i> (mg/kg i.p.)			
100	30	42.8 ± 2.3	60.28
200	30	26.2 ± 8.9	36.90
400	30	24.2 ± 4.9	34.08
ASA (mg/kg i.p.)			
100	30	0.6 ± 0.6	0.85
Control	60	57.4 ± 6.4	100.00
<i>S. bicolor</i> (mg/kg i.p.)			
100	60	35.0 ± 5.4	60.98
200	60	13.6 ± 3.5	23.69
400	60	6.0 ± 1.8	10.45
ASA (mg/kg i.p.)			
100	60	2.6 ± 1.7	4.53
Control	90	45.8 ± 9.4	100.00
<i>S. bicolor</i> (mg/kg i.p.)			
100	90	20.8 ± 6.9	45.41
200	90	5.4 ± 3.4	11.79
400	90	12.2 ± 6.5	26.64
ASA (mg/kg i.p.)			
100	90	10.0 ± 1.6	21.83
Control	120	32.8 ± 3.5	100.00
<i>S. bicolor</i> (mg/kg i.p.)			
100	120	9.8 ± 0.9	29.88
200	120	15.4 ± 2.5	46.95
400	120	6.4 ± 1.0	19.51
ASA (mg/kg i.p.)			
100	120	5.8 ± 0.4	17.68

**Appendix VI:** Effect of methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg i.p.) on force-induced pain in mice

<i>Treatment</i>	<i>Tail Flick Force (gram) ± SEM</i>				
	<i>0 min</i>	<i>30 min</i>	<i>60 min</i>	<i>90 min</i>	<i>120 min</i>
Control	381.4 ± 48.0	405.6 ± 68.6	409.2 ± 65.9	361.2 ± 26.8	345.0 ± 30.3
<i>S. bicolor</i>					
100 mg/kg i.p.	446.4 ± 61.6	455.4 ± 88.4	430.2 ± 59.8	415.8 ± 61.9	381.6 ± 38.4
200 mg/kg i.p.	446.7 ± 54.1	507.6 ± 60.5	645.0 ± 38.6*	541.4 ± 36.8*	491.0 ± 56.0*
400 mg/kg i.p.	335.6 ± 36.8	420.0 ± 31.8	542.8 ± 39.3	412.5 ± 65.2	470.8 ± 52.1*
ASA					
100 mg/kg i.p.	365.3 ± 33.7	581.3 ± 72.8*	600.8 ± 48.1*	490.3 ± 55.2	486.0 ± 30.2*

\* = P<0.05; statistical difference from control (ANOVA; Student t-test).

**Appendix VII:** Effects of the aqueous and ethylacetate fractions (100 – 400 mg/kg i.p.) of *S. bicolor* leaf base extract on force-induced pain in mice

<i>Treatment</i>	<i>Time (min)</i>				
	<i>0</i>	<i>30</i>	<i>60</i>	<i>90</i>	<i>120</i>
Control	580.0 ± 129.0	705.0 ± 176.5 [125]	650.0 ± 202.7 [70]	759.0 ± 123.4 [179]	700.0 ± 170.9 [120]
Aqueous fraction:					
100 mg/kg i.p.	490.0 ± 108.5	640.0 ± 102.3 [115]	675.0 ± 135.0 [185]	793.0 ± 119.5 [303]	945.0 ± 24.3 [455]
200 mg/kg i.p.	380.0 ± 29.4	625.0 ± 164.4 [245]	765.0 ± 117.3 [385]	760.0 ± 132.9 [380]	461.0 ± 87.8 [81]
400 mg/kg i.p.	505.0 ± 145.5	690.0 ± 81.9 [185]	573.0 ± 148.6 [68]	1000.0 ± 0.0 [495]	805.0 ± 89.6 [300]
Ethylacetate fraction:					
100 mg/kg i.p.	420.00 ± 100.3	500.0 ± 62.2 [80]	350.0 ± 23.8 [-70]	365.0 ± 27.5 [-55]	340.0 ± 60.6 [-80]
200 mg/kg i.p.	410.0 ± 57.4	645.0 ± 143.8 [235]	560.0 ± 149.4 [150]	600.0 ± 116.9 [190]	560.0 ± 167.3 [150]
400 mg/kg i.p.	675.0 ± 68.5	760.0 ± 149.9 [85]	740.0 ± 101.7 [65]	812.0 ± 109.5 [137]	856.0 ± 97.2 [181]
ASA					
100 mg/kg i.p.	380.0 ± 42.4	630.0 ± 95.4 [250]	770.0 ± 125.8 [390]	536.0 ± 130.7 [156]	515.0 ± 61.8 [135]

Note: [ ] = The difference between the force borne by mice at zero minute and at all the time intervals.

**Appendix VIII:** Concentration dependent effect of methanolic extract of *S. bicolor* leaf base (0.04 – 2.56 mg/ml) on isolated rabbit jejunum. (Sensitivity = x 3mV; speed = 24 mm/min; Ach = acetylcholine, 0.004 – 0.016 µg/ml)

**Appendix IX:** Relaxation effect of 70 % methanolic leaf base extract of *S. bicolor*  
(0.04 – 5.12 mg/ml) on rabbit jejunum

<i>Organ bath concentration (mg/ml)</i>	<i>Log organ bath concentration</i>	<i>Maximum Height of Relaxation (mm)</i>				<i>Average Height (mm) ± SEM</i>	<i>% of Maximal Relaxation</i>
		<i>H<sub>1</sub></i>	<i>H<sub>2</sub></i>	<i>H<sub>3</sub></i>	<i>H<sub>4</sub></i>		
0.04	-1.4	20.0	18.0	13.0	9.0	15.0 ± 2.5	0.00
0.08	-1.1	14.0	11.0	12.0	8.0	11.3 ± 1.3	24.7
0.16	-0.8	13.0	9.0	11.0	5.0	9.5 ± 1.7	36.7
0.32	-0.5	4.0	7.0	5.0	3.0	4.8 ± 0.9	68.0
0.64	-0.2	3.0	4.5	1.0	1.0	2.4 ± 0.9	84.0
1.28	0.1	0.0	-1.0	-1.0	0.0	-0.5 ± 0.3	103.3
2.56	0.4	0.0	-1.0	0.0	0.0	-0.3 ± 0.3	102.0
5.12	0.7	-1.0	-1.0	0.0	-	-0.7 ± 0.3	104.7

**Appendix X:** Non-myogenic effect of methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) on isolated guinea pig ileum. (Sensitivity = x 3 mV; speed = 24 mm/min; Ach = acetylcholine (0.004 – 0.016 µg/ml)).

**Appendix XI:** Slight relaxation effect of methanolic extract of *S. bicolor* leaf base  
(1.28 – 5.12 mg/ml) on guinea pig ileum. (Sensitivity ...)

**Appendix XII:** Contraction effect of 70 % methanolic leaf base extract of *S. bicolor*  
(0.04 – 5.12 mg/ml) on rat stomach fundus strip



**Appendix XIII:** Contraction effect of 70 % methanolic leaf base extract of *S. bicolor*  
(0.04 – 5.12 mg/ml) on rat stomach fundus strip

<i>Organ bath concentration (mg/ml)</i>	<i>Log organ bath concentration</i>	<i>Maximum Height of Contraction (mm)</i>		<i>Average Height (mm) ± SEM</i>	<i>% of Maximal Contraction</i>
		H <sub>1</sub>	H <sub>2</sub>		
0.04	-1.4	24.0	3.0	13.5 ± 10.5	51.9
0.08	-1.1	23.5	4.0	13.8 ± 9.8	53.1
0.16	-0.8	26.0	6.0	16.0 ± 10.0	61.5
0.32	-0.5	28.5	7.0	17.8 ± 10.8	68.5
0.64	-0.2	27.0	5.0	16.0 ± 11.3	61.5
1.28	0.1	31.0	5.0	18.0 ± 13.0	69.2
2.56	0.4	31.0	10.0	20.5 ± 10.5	78.8
5.12	0.7	29.0	23.0	26.0 ± 3.0	100.0

**Appendix XIV:** Effect of methanolic extract of *S. bicolor* leaf base (0.4 – 3.2 mg/ml)  
on isolated rat atria ....

**Appendix XV:** Effect of methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) on isolated rat portal vein .....

**Appendix XVI:** Effect of methanolic extract of *S. bicolor* leaf base (0.04 – 5.12 mg/ml) on isolated rat uterus .....

**Appendix XVII:** Effect of methanolic extract of *S. bicolor* leaf base (0.64 – 2.56 mg/ml) on oxytocin-induced contraction of isolated rat uterus .....

**Appendix XVIII:** Effect of methanolic extract of *S. bicolor* leaf base (0.4 – 25.6 mg/ml) on isolated rat vas deferens .....

**Appendix XIX:** Antagonism between atropine (0.4 µg/ml), acetylcholine (0.16 µg/ml) and methanolic extract of *S. bicolor* leaf base (3.2 mg/ml) on rat vas deferens ....

**Appendix XX:** Antagonism between mepyramine (0.16  $\mu\text{g/ml}$ ), acetylcholine (0.16  $\mu\text{g/ml}$ ), and methanolic extract of *S. bicolor* leaf base (3.2 mg/ml) on rat vas deferens