INDUCTION OF MAMMARY TUMOUR IN ALBINO RATS BY 7,12-DIMETHYLBENZ(a) ANTHRACENE SUSPENDED IN TWO VEGETABLE OILS

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BBLLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AVARD OF THE DEGREE OF MASTER OF SCIENCE

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DECEMBER, 1990

DECLARATION

I hereby declare that this is a record of my own research and has not been submitted for the award of a degree at any other university. Information and excerpts from both published and unpublished work of others have been acknowledged in the text.

Omage Jebba James

CERTIFICATION

This thesis entitled "Induction of Mammary Tumour in albino rats by 7,12-Dimenthylbenz(a)anthracene suspended in two vegetable oils" by Omage Jebba James meets the regulations governing the award of the degree of M.Sc. in Biochemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to Mrs. Christiana Omage for her love and devotion, my brothers and sisters for their sincere prayers and to JEHOVAH for the uncountable things he has done in my life.

AKNOWLEDGEMENTS

I wish to express my profound gratitude and indebtedness to Dr. J.A. Elegbede, the supervisor of this work. His practical experience in tumourigenesis and great interest in this work, very valuable guidance, useful suggestions, assistance and constructive criticisms made this thesis a success. May God reward him abundantly.

I am exceedingly grateful to Dr. G.H. Ogbadu (Head of Department) and Mr. M. Owoshagba (Chief technologist) for making available a carcinogen room for this study. I acknowledge the co-operation and help of all members of the department of Biochemistry particularly Mr. E. Kolawole, Mr. George, Mr. Sunday Awotele. Mr. J.J. Elisha and Mr. Ibrahim throughout the duration of this work.

I am exceedingly grateful to Professor E.O. Gyang for the significant role he has played in my life. I am grateful to Professor C.O. Njoku for interpreting my slides. and Dr. K A.N. Esievo for the interest he showed in the work. I also appreciate the individual roles Professor C.O. Ikediobi, Dr. A.I. Ukoha, Dr. O. K. Obagaiye, Mr. I.I. Achoba, Mr. J.N. Andrew and Ms. E. Onyike, played during the course of this programme.

My warmest regards goes to the memory of my father, M.J.I. Omage for an accountable life. I am grateful to my mother, Mrs. C. Omage for making so much sacrifice to see me through. I am also grateful to my uncle Capt. B.I. Akhigbe for his moral support and the members of Omage family, particularly C.W.O. Godwin Omage, Solomon Omage, Robert Omage, Fred Omage, Juliana Omage. Mrs. Eunice Molokwu and Mrs. Esther Ajayi who

were helpful and reassuring right from the beginning of my course. I quite appreciate your individual efforts. I also acknowledge the contributions of Josephine Oweh (Mrs), Jerry Omage, Rufus Omage and Joseph Omage.

Many thanks to my friends and colleagues, Akeem Alimi, Wilfred Wiggle, Augustina Duru, F.U. Ebele (Mrs), Charles Boboe, P.C. Onyenekwe, Bukky Akinyemi, Ben Chukwujekwe, Jideofor Udeke, R. Aliyu (Mrs), Doris, Martina, James Okoro, Ngozi Oburo, Bello Abubakar, and Jude Oha. Your contributions will long be remembered. I am very grateful to Mrs F. Oladipo and Mr Titus for the wonderful work they did in typing this thesis.

Finally, I give all praise to JEHOVAH JIREH who knows exactly what it takes to do a postgraduate programme at home.

ABSTRACT

The susceptibility of female albino rats to mammary tumour induction by 7, 12-di-methylbenz(a) anthracene (DMBA) dissolved in two types of oils was investigated. Female albino rats (aged 54 days) were intubated with BKBA (65mg/>;g body weight) dissolved in either sesame seed oil (Group A) or soyabean oil (Group B). The Influence of DMBA on male rata was alao investigated by intubating mole albino rate with DMBA in soyabean oil (Group C) or sesame seed oil (Group D). The animals were fed laboratory animal chow (Pfizer-Ladokun feed), weighed weekly and the females were palpated for mammary tumour development starting 3 weeks post intubation. At 42 weeks, all surviving animals were killed by decapitation,. At necropsy, blood and tissue samples were taken for biochemical analysis, haemntological studies and histopnthological examination.

In the females, biochemical profile shows significantly ($P < 0.05$) higher levels of total serum protein and total serum cholesterol in the soyabean oil group than the sesame seed oil group. The liver weight was also significantly ($P < 0.05$) higher in the soyabean oil group than the sesame seed oil group. *to* significant ($P > 0.05$) differences were observed between the two groups in growth rate, body weight gain, haematocrit, haemoglobin and lymphocyte counts.

In the males, biochemical profile shows significantly ($P < 0.05$) higher levels of total serum protein and total serum cholesterol in the soyabean oil group than the sesame seed oil group. There was significant ($P < 0.05$) difference in body weight gain and growth pattern between the two treatment groups. There was significantly ($P < 0.05$>

higher scrotal sac and liver weights in the sesame seed oil group than the soyabean oil group. There was significant <P < 0.05) difference in the haematocrit, and haemoglobin levels while no differences (P > 0.05) were observed in tne lymphocyte counts of both treatment groups.

While soyabean oil may be substituted for sesame seed oil as DMBA solvent, the oil and the use of albino rats as a model for mammary tumourigenes and scrotal sac development requires further investigation.

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CHAPTER ONE

INTRODUCTION

Breast cancer is one of the major cancers of women, representing approximately 27% of all cancers identified in women (Gould et al.,1989). Daoud et al. (1968) reported the incidence of breast cancer to be above one quarter of all female malignancies in Nigeria, Sudan, Uganda and most tropical countries. Incidence rate of 21.4% (Elmes and Baldwin 1947), 16.8% and 15.3% (Miller, 1987) had been reported for Nigeria, 29.7% for Sudan (Daoud et al., 1968) and 9.3% of all malignancies in Ugandan women (Templeton, 1973). Breast cancer is also a major health problem affecting approximately one in ten American women (Moore et al., 1988).

Cancer is a chronic pathological endpoint of a prolonged complex process and by definition involves cells which divide when they should not, usually to produce the contiguous cellular masses called tumours. It is as a result of exogeneous or endogeneous stimuli interacting with cells of a genetically susceptible host and this interaction could be influenced by a myriad of factors that either enhance or retard the reaction (Shimkin 1981). Among the factors related to the host are age, sex, nutritional and immunological status, other diseases and psychological influences (Shimkin 1981). Among the initiating factors are the dose and concentration of carcinogen and route of administration. The balance between the stimulus and the host determines the proportion of neoplasms evoked and the rate at which they appear (Shimkin, 1981).

Different classes of cancers are known and they arise by changes in the various forms of differentiated cells with the resulting cancer

cells exhibiting many of the morphological and functional characteristics of their normal precursors (Watson, 1975). Thus the term carcinoma refers to malignant tumours of epithelial origin, while sarcoma are those arising from connective tissue cells; papilloma and adenoma are benign growths. Hepatomas, cholangiomas and reticulum cell sarcomas are all tumours peculiar to the liver.

Most organs and tissues of the body are capable of exhibiting cancerous growth. These growths may be localised to the organ in which they arise and are said to be benign. Cancer is also characterized by a tendency to spread locally from its source and to metastasize to distant parts of the body; damaging or destroying the normal tissues it invades.

A variety of factors may initiate or induce cancer. These include genetic influences (Lynch et al., 1977; Doll and Peto, 1981); chemical carcinogens (Rakin et al., 1968; Huggins, 1979; Keller, 1967; Doll, 1977; Elegbede et al., 1984, 1986a, Elson et al., 1988; Gould et al., 1989), hormones (Ziel and Finkle, 1975; Smith et al., 1975; Cole, 1977; Poskanzer and Herbst, 1977; Miller, 1978; Worland et al., 1989), and radiotherapy (Mackenzie, 1965; Archer, 1977; Boice and Monson, 1976; Hrubec et al., 1989).

The best way of controlling cancer is prevention which can be achieved by:

(a) understanding the basic mechanism(s) of carcinogenesis;

(b) identifying and removing carcinogens from the environment;

(c) developing new therapies and increasing the therapeutic ratio of current modalities; and

(d) identifying dietary anticarcinogens.

Induction of mammary tumours by DMBA in female rats is an experimental model used in breast cancer studies though laboratory rats differ in their susceptibilities to mammary carcinogenesis with the Copenhagen rat being completely resistant (Gould et al., 1989). The more commonly used model is that developed by Huggins (1979) in which a suspension of DMBA (130mg/kg body weight) in sesame seed oil was given to Sprague-Dawley rats orally. This treatment induced mammary tumours in greater than 95 per cent of animals within a period of 3 to 6 months (Huggins 1979). Though other carcinogens induce mammary tumours in rats, either repeated large doses are required or the time interval necessary for the neoplasm to develop is long (Wattenberg, 1978).

Some of the problems facing experimental cancer research in Nigeria include:

(i) inadequate knowledge of the susceptibility of available strains of rats to mammary tumour induction by DMBA

(ii) relative scarcity of unadulterated sesame seed oil;

(iii) inadequate research facilities.

Sesame seed oil and soyabean oil are similar in their contents of saturated fatty acids and total unsaturated fatty acids though they differ in their contents of monounsaturated and polyunsaturated fatty acids. Moreover, soyabean oil is also readily available in our local markets. This study was therefore embarked upon to:

1. investigate the susceptibility of locally available strain of albino rats to mammary tumour induction by DMBA;
2. investigate if soyabean oil can be substituted for sesame seed oil as a medium in preparing DMBA suspension for oral intubation;
3. investigate the influence of DMBA on male albino rats.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 NUTRITION AND CANCER

In recent years there has been interest in a possible relationship between diet and cancer. The recent accumulation of epidemiologic and laboratory evidence in man and animals shows with some certainty that dietary and nutrient excesses, deficiencies or imbalances are related to the development of cancers in the oesophagus, stomach, colon, pancreas, liver, endocrine organs and breast (Enig et al., 1978; Anon., 1975; Lowenfels and Anderson, 1977; Armstrong and Doll, 1975; Alcantara and Speckmann, 1976; Hankin and Rawlings, 1978; Reddy et al., 1980).

Involvement of nutrition in the aetiology of cancer is derived primarily from epidemiological studies and a number of laboratory animal studies. Cairns (1975) for instance estimated that 90% of cancers in man are epithelial carcinomas that are closely related to environmental causes and involve sites related to the portal of entry of or exposure to environmental agents, while Wynder and Gori (1977) estimated that over one half of all female cancer deaths and 30% of all male cancer deaths may be related to nutritional factors.

One of the intimate contacts with the environment is through food. Diets furnish proteins, carbohydrates and fats as the bulk energy components, each of which also supplies important building blocks such as amino acids, monosaccharides and fatty acids for organ and cell structures etc. In addition essential vitamins and minerals are taken in for optimal utilization and good health. Apart from the basic nutrients there are also non-nutritive substances which may have beneficial or harmful effects (Petering, 1978; Chasseaud, 1979).

Cancer mortality depends on many related factors in which nutrition may play an important role. According to Vitale (1975) and Petering (1978), diet and nutrition can affect several steps in carcinogenesis directly or indirectly by influencing metabolic processes (Fig. 1). Nutritional influences may occur in 3 ways:

(a) Nutrients, food additives or contaminants acting as full carcinogens or as initiators, promoters or accelerators in carcinogenesis process.

(b) Dietary deficiencies (macro-micronutrients) leading to biochemical malfunctions that in turn initiate neoplastic process;

(c) nutritional excesses (calories, macronutrients) inducing a variety of metabolic abnormalities that in turn promote cancer.

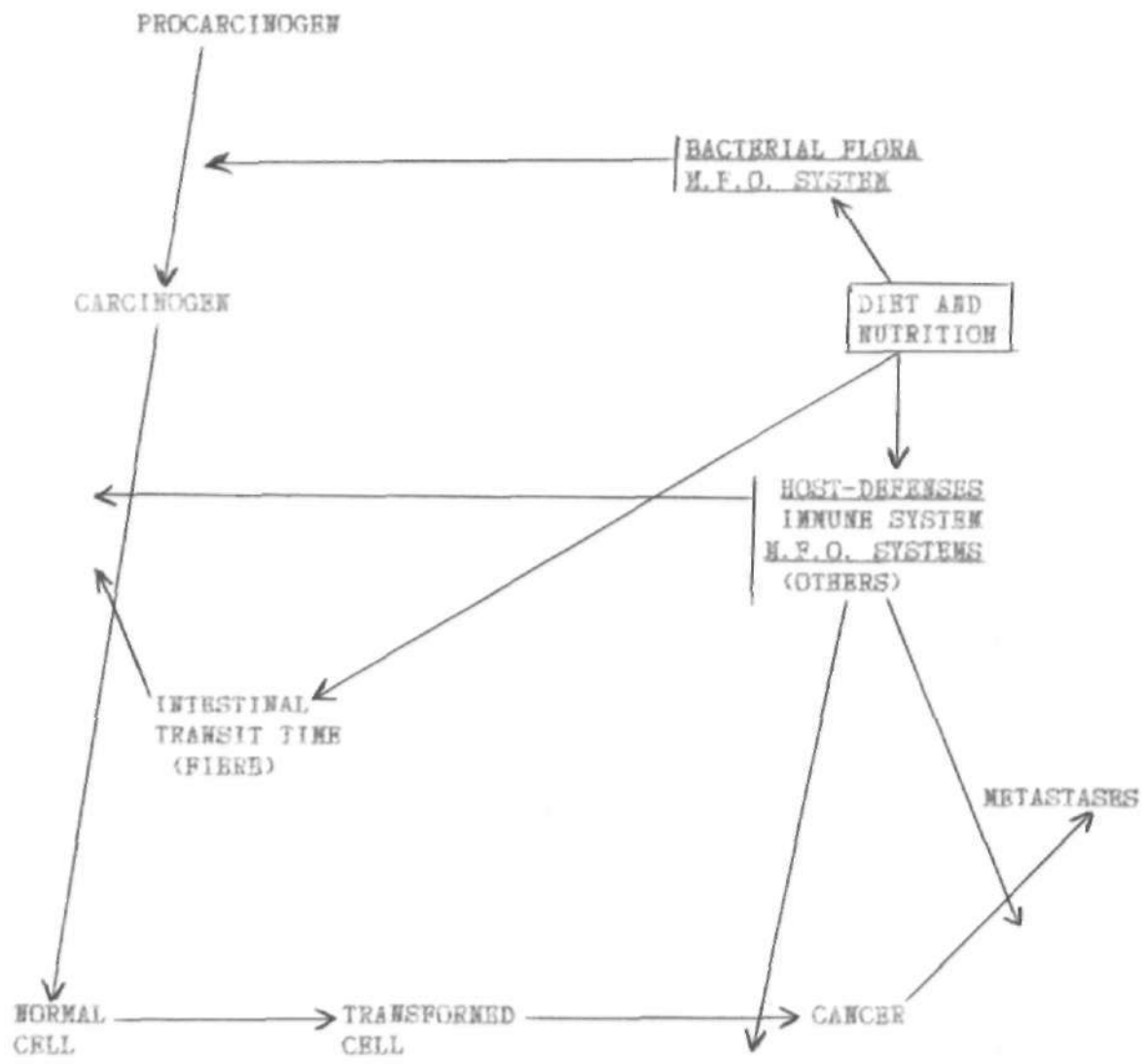
Vitale (1975) reported that nutrients or diets must exert their major effects in one of several ways:

(a) as carriers of a procarcinogen or carcinogen (for example, nitrates in meat).

(b) by altering the intestinal flora that act on procarcinogens;

(c) by increasing the level of cocarcinogens such as certain fatty acids and sterols;

Figure 1: Schematic diagram showing the multistep process involved in chemical carcinogenesis



Arrows indicate the steps that can be affected by diet and nutrition directly or indirectly.
Source: Extracted from; Petering, H.G (1978). Diet, Nutrition and Cancer: In Inorganic and Nutritional Aspects of Cancer (Schrauzer G.N. ed.) Plenum Press. New York and London pp.207-228.
M.F.O = Mixed Function Oxidase

(d) by altering tissue morphology so that it is more susceptible to the action or penetration of carcinogen as well as the cocarcinogen (for example diets that increase the concentration of sterols in the intestinal lumen and that have been implicated in enhancing chemical carcinogenesis); or (e) by influencing the host defense systems or one of its components, rendering the animal and/or organ more susceptible to both the chemical carcinogen and procarcinogen, be it dietary or endogenously derived.

Environmental factors are more important causes of most types of cancer than inheritance. It is difficult however to establish whether there is a threshold level for a carcinogen in the environment below which there is no risk (Anon., 1978). At low levels of an environmental hazard the spontaneous rate of cancer development and variations in individual susceptibility seems to complicate the picture (Anon, 1978).

Cancer incidence varies worldwide and tends to change with migration. There have been a number of indications from epidemiological observations that some types of cancer occur more frequently in societies with certain dietary habits than among other groups of people (Reddy et al., 1980). For example, among Japanese and West African populations, there are cancer patterns that are greatly different from those found in natives of those areas who have migrated to the United States (Lijinsky, 1983; Dunn, 1975; Haenszel and Kurihara, 1968). For example, gastric cancer is reported to be lower in Japanese living in Hawaii than that recorded for Japanese living in Japan. The Scandinavian countries show the next highest incidence followed by the United Kingdom, Chile, Colombia, Puerto Rico and Israel. However in Israel a higher incidence was noted in those of European extraction as

compared with those whose origins were in Africa or Asia (Edington and Gilles, 1976). Dunn (1975) reported a lower incidence of gastric cancer in the United States compared to Japan. The incidence in Africans living in Africa is low excluding perhaps the Kivu province in the Congo, whereas the incidence in South African coloureds, whites and Asians far exceeds the incidence in the United States being highest in the coloured in both sexes (Edington and Gilles, 1976). In Nigeria, the incidence of gastric cancer is similar to that seen in the United States until the age of 50 years and is then very much less in the older groups.

Cancer of the breast shows geographical variations of incidence which also suggest that there is a nutritional component in its aetiology (Anon, 1978; Hankin and Rawlings, 1978). Generally, incidence and mortality rates in North America and nothern Europe are five to six times higher than those in most countries of Asia and Africa. Intermediate rates prevail in southern Europe and South America (Hankin and Rawlings, 1978; Zemla, 1984). Cancer of the oesophagus varies more strikingly in different communities than that of any other part of the body. This condition is common in Chile, Assam, Jamaica, Curacao, with the highest rates in the world in northern Iran and in parts of East and South and Central Africa (Anon, 1978). The relative infrequency of cancer of the oesophagus in West and North Africa is remarkable. In South Africa, whites and Asians are not affected. In the United States, there are substantial racial and geographical variations. There is a rising non-white mortality of all ages and it is relatively stable in the white population (Schoenberg et al., 1971). High incidence rate of

liver cancer has been reported in the Yorubas of Western Nigeria (Edington and Maclean, 1965).

The relationship between diet, nutrition and cancer is complex. Different types of cancers are not necessarily affected by dietary components in the same manner, since the development of certain neoplasms may be repressed by specific dietary deficiencies while others are potentiated by such deficiencies (Alcantara and Speckmann, 1976).

Many investigators (Miller et. al. 1976, Lowenfels and Anderson, 1977, Phillips and Snowdon, 1983) have indicated that diet composition is associated with differences in mortality associated with cancer . Certain dietary habits correlate very strongly with the incidence of several forms of cancer. Cancer of the colon and cancer of the breast are related to increased dietary fat intake (Wynder and Shigematsu, 1967; Carroll, 1975; Miller et al., 1978; Kalamegham and Carroll, 1984; Reddy et al., 1980) and more specifically to increased animal fat intake (Hentges et al., 1977; Reddy et al., 1974, 1977, and 1980; Wynder et al., 1969; Wynder and Reddy, 1975; Carroll, 1975; and Kolonel et al., 1983) and polyunsaturated fatty acids (PUFA) (Pearce and Dayton, 1971; Kolonel et al., 1983).

Furthermore, examination of epidemiological data on human populations has shown positive correlations between breast cancer mortality and the intake of certain non-lipid components such as animal protein and simple sugars (Berg, 1975, Drasar and Irving, 1973; Kolonel et al., 1983; Hoehn and Carroll, 1979) whereas no such correlations are seen with intakes of vegetable protein or complex carbohydrates.

Case control and prospective studies indicate that both cancer of the breast and cancer of the corpus uteri are associated with obesity

(Lin et al., 1971; De Waard and Baanders van Halewijn, 1974). Actuarial records (Tannenbaun, 1940)indicate that obese or overweight men are more liable to cancer than are normal or underweight men.

In experimental animals, both spontaneous and/or chemically induced mammary tumours are also influenced by diet high in unsaturated fatty acids (Gammal et al., 1967; Chan and Cohen, 1974; Rogers and Wetsel, 1981; Sylvester et al., 1986; Sundram et al., 1989), polyunsaturated fat (Katz and Boyland, 1986; Cave and Jurkowski, 1986) animal fat (Sylvester et al., 1986) and increased dietary protein (Hawrylewicz et al., 1986). Underfeeding reduces the incidence of both spontaneous and induced tumours in experimental animals (Tannenbaun, 1942) while caloric restriction slightly retard the growth of established mammary tumours (Tannenbaun and Silverstone, 1953) hence it was concluded that the relevant effect of dietary or caloric restriction was probably mediated through its effect on body weight.

A number of factors have been demonstrated to increase a woman's risk for the development of breast cancer. Diet may promote or inhibit breast cancer through its effects on hormonal systems. Menarche at an early age, first birth at a late age, low parity, benign breast disease and a family history of the disease each double the risk (Marubini et al., 1988; Anderson and Badzioch, 1989; Hankin and Rawlings, 1978). There is circumstantial evidence linking breast and uterine cancer to estrogens and particular events of early life e.g. patterns of hormone metabolism may be determinants of breast cancer risk (Hankin and Rawlings, 1978; Anon, 1978). For instance girls who grow fast and start menstruating early have a slightly greater chance of developing breast cancer.

The level of nutrition and/or dietary fat predisposes to breast cancer by changes in the balance of hormones e.g. production of different proportions of estrogens (Anon, 1978; Reddy et al., 1980; Hankin and Rawlings, 1978). The need of estrogens for tumour induction in the mouse has been demonstrated (Muhlbock, 1956).

In man the diminution of risk for the development of breast cancer afforded by early oophorectomy supports a permissive role for estrogens (Feinleib, 1968; Hiroyama and Wynder, 1982) thus estrogens are considered to be carcinogenic to human breast also (Cole and Machalon, 1969). Women produce substantial amounts of three major estrogens: estrone (E1), estradiol (E2) and estriol (E3). E1 and E2 have been shown to cause cancer in rodents (Mackenzie, 1955; Silberberg and Silberberg, 1956; Hankin and Rawlings, 1978). Prolactin, a pituitary peptide, also acts as promoter in mammary tumourigenesis in rodents (Furth, 1972; Welsch and Nagasawa, 1977). Studies demonstrates that in mammary tumourigenesis in a given strain of rat, the tumour incidence corresponds directly to serum prolactin titres (Meites, 1972; Sinha et al., 1973; Welsch et al., 1970). In vivo however, prolactin alone, in the absence of estrogen cannot sustain growth of rat mammary tumours (Dao and Sinha, 1972). A certain basal level of estrogen is required for maintenance (Leung et al., 1975). Paradoxically however, whereas estrogen at low concentrations act synergistically with prolactin and stimulate mammary tumour growth, at high concentrations it antagonizes the action of prolactin and inhibits mammary tumour growth (Huggins, 1965; Nagasawa and Yanai, 1971; Welsch and Nagasawa, 1977). Although the influence of these two hormones on tumour growth has been

demonstrated, the intimate relationship of these two hormones in causing tumour growth is not well understood (Leung et al., 1975).

Diet and nutrition are viewed more appropriately as modifiers, rather than initiators of tumourigenesis. Cancer rates seem to be influenced by two counter-active types of dietary agents:

(i) carcinogens that cause increasing rates for increasing exposure; and

(ii) anticarcinogens, that reduce the effects of the carcinogen.

The consequence to the host will depend on the balance between these opposing forces (Alcantara and Speckmann, 1976).

2.2 AETIOLOGY OF CARCINOGENESIS

Carcinogenesis is a multistage process involving two discontinuous stages (Foulds, 1975). This has been demonstrated in experiments mainly with skin tumours (Berenblum and Schubik, 1947). The first stage, initiation, is defined as cellular lesions that are induced rapidly by carcinogenic insult, and are persistent and heritable (Gould et al., 1987). It involves the carcinogenic event in which normal cells are transformed into neoplastic cells by interaction with a carcinogenic agent (Hopkins and West, 1976). The second stage is referred to as promotion/progression and represents a continuum of events leading from a single initiated cell to the heterogeneous neoplasm that results in the death of its host (Gould et al., 1987; Hopkins and West, 1976). It has been suggested that it is during this second stage that many of the known effects of environmental factors such as dietary fat (Hopkins and West, 1976), protease inhibitors (Kennedy and Little, 1981), d-limonene (Maltzman et al., 1989; Elson et al., 1988; Gould et al. 1986)

selenium (Thompson and Becci, 1980). which influence carcinogenesis are felt. This stage may also involve a progressive selection of cell populations for characteristics such as autonomous growth, malignancy and resistance to cytotoxicity (Hopkins and West, 1976).

The aetiology of breast cancer is complex and not clearly defined. Several factors are known to play significant roles in the causation of cancer. Valuable information regarding both aetiology and progession of cancer have been obtained from the characterization of clonal cytogenetic changes. Many of these cytogenetic changes have been associated with the activation of specific oncogenes (De Klein et al., 1982; Dalla Favera et al., 1982) or loss of suppressor gene function (Cavenee et al., 1983).

2.3 FAT EFFECT: MECHANISM OF ACTION

Several hypothesis on the possible mechanism(s) by which dietary fat may exert its effect on breast cancer have emerged (Hopkins and West, 1979; Hankin and Rawlings, 1978; Alcanatara and Speckmann, 1976). They fall into 2 basic categories namely. those involving direct effects of fat on tumour development and those involving indirect effects on host metabolism (Reddy et al., 1980; Cohen, 1981).

(a) Direct Effects: This model involves changes in the lipid content of cell membrane, the formation of lipid peroxides and the synthesis of prostaglandins (PG) (biologically active derivatives of the essential fatty acids, arachidonate and linoleate). Adipose tissues rapidly take up and slowly release lipid-soluble agents. Beer and Billingham (1978) proposed that the adipose tissue surrounding ductal cells may contribute to the susceptibility of tissue cells to cancer by prolonged exposure to lipid-soluble carcinogens of endogenous or exogenous origins. Dietary

fats may alter the distribution or influence the metabolism of chemical carcinogens (Hopkins and West, 1976). It is also possible that increased peroxidation of membrane lipids results in alterations in the function of transformed mammary cell membranes which in turn, permit increased rates of growth (Hopkins and West, 1976). Dietary fat influences the rate of mammary nodulogenesis and subsequent tumour formation (Faulkin et al., 1986) while linoleate promotes tumour growth (Mehta et al., 1986). Various properties of membranes are influenced by the fatty acid composition of membrane lipids which was reported to be markedly altered by feeding coconut oil or corn oil (Gammal et al., 1967) or to be unstable when the fat intake of the host was altered (Rao and Abraham, 1976). Tumour tissues tend to have greater proportions of PUFA than normal glands, this according to Rao and Abraham (1976) suggests that tumour growth is related to arachidonic acid availability through either prostaglandin synthesis or unknown membrane function. Prostaglandins act as intracellular regulatory molecules that together with the cyclic adenosine monophosphate (cAMP) systems, govern the response of cells to hormonal stimulation (Reddy et al., 1980).

(b) Indirect mechanisms involve (i) sub-version of the immune system; (ii) stimulation of mixed function oxidase systems involved in carcinogen activation; (iii) alterations in fecal flora and bile acid metabolism; and (iv) alterations in endocrine milieu of the host (Cohen, 1981). Increased amount of dietary PUFA may suppress the cell-mediated immune rejection mechanism, either directly or after conversion to PG and thereby enhance the capacity of transformed cells to proliferate (Hopkins and West, 1976). Fats also cause decreased hepatic microsomal enzyme activity and influence DNA repair potential (Hopkins

and West, 1976). Fats may also be involved in altering the hormonal environment (Berg, 1975; Wynder et al., 1971). Chan and Cohen (1975) observed that dietary fat promotes mammary tumour development by increasing the concentration of circulating prolactin relative to that of estrogen. All these factors provide possible mechanisms by which dietary fat influences tumourigenesis.

2.4 RODENT MODELS FOR MAMMARY CARCINOGENESIS

Rodent mammary models have been used to study general as well as organ-specific problems in carcinogenesis (Gould et al.,1989). Spontaneous and induced mammary tumour models in both mice and rats have been valuable (Gould et al., 1989). The rat, is a better model of the human neoplasm in that unlike the mouse model it does not have a known viral aetiology but has a similar hormonal response pattern closely resembling that found in human breast carcinomas (Gould et al., 1989).

A wide variety of inbred laboratory mice (Mus musculus) is available for study. Strains such as C3H, A, DBA, and BALB/cf C3H are known to have a greater than 70% lifetime incidence of developing both spontaneous and induced mammary tumours in breeding mice while a variable incidence of both spontaneous and induced mammary tumour is observed in virgin mice. Other strains such as C3Hf/He and 020 mice have moderate incidences (25-50%) of mammary neoplasms, whereas others such as BALB/c, C57BC, C3H/st Wi, AR and DBA/2f are considered to be resistant to both spontaneous and induced cancers (Gould et al., 1989).

The rat is a major experimental animal in transplantation, immunology, genetics, cancer research, pharmacology, physiology, neurosciences and aging (Gill et al., 1989). The strains and randomly

bred stocks that have been used almost exclusively are derived from the Norway rat (Rattus norvegicus). The rat is a useful model for the investigation of mammary cancers. The Huggins protocol for the induction of mammary tumour in female rats has been widely used in breast cancer studies (Eskin et al., 1968; Huggins, 1979; Elegbede et al., 1986 a & b; Elson et al., 1988). The biology of the mammary tumours produced by the protocol resembles the biology of human breast cancer (Gould et al., 1989).

Many inbred and outbred rat strains are available but they differ in their susceptibilities to mammary carcinogenesis, through genetic regulation (Gould et al., 1989). Susceptibilities can be divided into 3 classes: high (Winster Furth WF, Sprague-Dawley SD, Lewis rats); low to intermediate (Fischer 344. F344, August,Aug., Long-Evans LE rats) and none or resistant (Copenhagen, cop rats) (Moore et al., 1988). The highly susceptible strains possess "enhancer genes" which enhance susceptibility to induced mammary cancer, while the Cop rats possess a single "suppressor gene" which confers complete resistance to mammary cancer induction. The intermediate to low strains have neither enhancer nor suppressor genes (Gould et al., 1989). Both the enhancer and suppressor genes may be operating at a later stage in the carcinogenesis process in mammary epithelial cells (Moore et al., 1988). The tumours induced in the susceptible strains can be divided into 3 groups based on whether they continue to grow, or stop growing and either become static or regress (Young and Cowan, 1963).

2.5 CHEMICALLY INDUCED TUMOURS

Several compounds have been shown to induce tumours in one or more species of experimental animals (Clayson, 1962). These compounds include polycyclic aromatic hydrocarbons (PAHs), the aromatic amines and azo compounds, the alkylating agents, and others. (Clayson, 1962). Among the PAHS, DMBA has been extensively used for tumour induction in the skin (DiGiovanni et al., 1986; Verma et al., 1978; Clark et al., 1984; Elegbede et al., 1986 b, c) salivary gland (Alam et al., 1984), mammary gland (Eskin et al., 1968; Huggins, 1979; Elegbede et al., 1984, 1986a; Das et al., 1989; Sundram et al., 1989; Sylvester et al., 1986; Elson et al., 1988) and reproductive tract (Cotchin and Marchant, 1977). 3-Methylcholanthrene, induces tumours in the mammary gland (Huggins, 1979; Miller, 1970)skin (Wattenberg, 1975), and the reproductive tract (Cotchin and Marchant, 1977). Benzo(a)pyrene (B(a)P) induces tumours in the skin (Cavalieri et al., 1986), pulmonary tract and liver (Varanasi et al., 1986) and reproductive tract(Cotchin and Marchant, 1977), while 7,8,12-trimethylbenz(a)anthracene (TMBA) induces tumours in the mammary gland (Huggins, 1979) and ovary (Cotchin and Marchant, 1977). N-methyl-N-nitrosourea (NMU) induces tumours in urinary bladder (Sporn et al., 1977), colon (Narisawa et al., 1981) and the mammary gland (McCormick and Moon, 1983; Maltzman et al., 1989; Gullino et al., 1975; Sukumar et al., 1983; Gould et al., 1989).

Some of these carcinogens for example. B(a)P acts at the initiation stage and is termed incomplete carcinogen while others like DMBA act both at the initiation and promotion stages of carcinogenesis and is termed complete carcinogen (Clayson, 1962; Das et al., 1989; Miller, 1981). Some of these carcinogen are direct-acting and do not require

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Some of these carcinogens for example. B(a)P acts at the initiation stage and is termed incomplete carcinogen while others like DMBA act both at the initiation and promotion stages of carcinogenesis and is termed complete carcinogen (Clayson, 1962; Das et al., 1989; Miller, 1981). Some of these carcinogen are direct-acting and do not require

metabolic activation to generate the key reactive intermediate for example. NMU. Others require metabolic conversion by the host to become active, and are labelled "procarcinogens" (Wattenberg, 1978; Weisburger and Williams, 1975; Miller, 1970; Maltzman et al., 1989). It has been proposed (Gould et al., 1982) that the induction of neoplastic transformation by PAH's occurs through chemical modification of critical cellular macromolecules such as deoxyribonucleic acid (DNA) by the reactive metabolites (Gould et al., 1982) more specifically to epoxides (arene oxides) (Heidelberg, 1975; Jerina and Daly, 1974; Das et al., 1989; Moore et al., 1988; Christou et al., 1987) free radicals (Tso et al., 1974) one electron oxidation to give radical cations (Fried, 1974) and the 6-hydroxymethylation of benzo(a)pyrene (Flesher and Sydnor, 1973). The reactive intermediates bind covalently to cellular macromolecules and this interaction is thought to be responsible for initiation of carcinogenesis (Moore al al., 1988; Moore et al., 1987; Gould st al., 1989; Weston et al., 1986).

The studies described in this thesis investigate the susceptibility of locally-available strain(s) of female albino rats to DMBA - induced mammary tumourigenesis and the effectiveness of soyabean oil as a solvent for preparing DMBA suspension.

CHAPTER THREE

MATERIALS AND METHODS

3.1 OIL EXTRACTION

2kg of sesame seed (Sesasum indicum L.) was purchased from local Zaria market in Kaduna State. The seeds were the early variety grown in parts of Kano State. The seeds were cleaned by washing thoroughly but briefly in water and sun-dried for two days. The dried seeds were roasted in an oven at 150°C for about 1 hour and then blended to a very fine oily paste.

The pressing out of the oil from the paste was achieved by using a hydraulic-jack-operated oil press fabricated by the Department of Agricultural Mechanization of the Institute of Agricultural Research (I.A.R), Samaru, Zaria. About 700mls of sesame oil was obtained.

Soyabean oil was purchased from local Zaria market in Kaduna State.

3.2 ANIMALS AND DIETS

The rats used were weanling albino rats (Rattus norvegicus) (Plate 1) bred in the Department of Biochemistry, Ahmadu Bello University, Zaria. The rats were randomized at 49 days of age into two treatment groups. The number and mean weights of rats in the groups are similar (13, 80kg each). The rats were individually ear-punched at 50 days of age. Water and pelleted laboratory animal chow (Pfizer-Ladokun feed) were provided ad Libitum throughout the experimental period.

Plate 1: An albino laboratory rat.



This adult female rat was placed on a dark background levelling table for photography.

3.3 TUMOUR INDUCTION

Tumours were induced by a single gastric intubation of a suspension of DMBA in either sesame seed oil (Groups A and D) or soyabean oil (Groups B and C) at 54 days of age according to the Huggins protocol (1979). The amount of carcinogen used (see Appendix I for carcinogen preparation) was reduced to 65mg DMBA/kg body weight to prolong average tumour latency.

Animals were divided into four groups andhoused in 33 x 55cm stainless steel top-tough polypropylene base rat cages (at 11 females group A, 4 females group B, 9 males group C and 2 males group D and 4 rats each for the control groups animals per cage). Water was supplied ad libitum in stainless steel nozzle water bottles. Wood shavings were used as the Litter material. The cages were cleaned twice weekly at which time fresh litter and water were provided.

The rats were weighed weekly and palpated (Appendix I) weekly starting 3 weeks after DMBA administration. The appearance of palpable tumours (1 x 1mm) was monitored and recorded.

The experiment was terminated 42 weeks after DMBA administration at which point surviving animals were necropsied. Blood was collected for biochemical and haematological analysis. The mammary glands (females) scrotal sacs (males) and livers were removed, weighed and stored for histopathological examination.

3.4 BLOOD COLLECTION

Just before necropsy, blood samples were taken from chloroform-anesthetized rats by heart-puncture technique. The anesthetized rats were partially dissected to reveal the base of the heart which is fairly

visible through the diaphragm and blood was collected by heart puncture using sterile needles and syringes (Plate 2). Samples for blood analysis were dispensed into vials containing EDTA (1mg/ml) as the anticoagulant. Samples for serum analysis were collected in centrifuge tubes, allowed to clot for 1 hour and serum prepared by centrifugation at 2000g for 20 minutes in the cold room. The uncoagulated blood samples were analyzed for hematocrit, hemoglobin and differential blood counts while serum was assayed for total serum protein and total serum cholesterol.

3.5 DETERMINATION OF PROTEIN

Total serum protein was determined by the biuret (Mokrasch and McGilvery, 1956) method. Aliquots (0.2ml) of serum was mixed with water (2.8ml) and assayed for protein (See Appendix II for details).

3.6 DETERMINATION OF TOTAL CHOLESTEROL

Total serum cholesterol was determined by the method of Zlatkis et al. (1953). Ferric chloride-acetic acid reagent (4.9ml) (Appendix II) was added to the samples (0.1ml) in a glass stoppered centrifuge tube. The contents of each tube was well mixed and allowed to stand for 10-15 minutes for the proteins to flocculate and then centrifuged for 5 minutes at 2000g. The clear supernatant fluid (2.5ml) was then transferred to glass stoppered centrifuge tubes. Ferric-chloride-acetic acid reagent (2.5ml) was added to another tube to serve as the blank. Cholesterol standard (200mg/dL) (2.5ml) was added to another centrifuge tube.

Concentrated sulphuric acid (1.5ml) was added from a burette to each tube, stoppered tightly and mixed by repeated inversion. The stopper

Plate 2: Abdominal viscera of rat *in situ*: Protocol for blood collection by Heart Puncture (HP)



The rat was placed under chloroform-anesthetics before dissection to maintain normal blood circulation.

was loosened carefully and the solutions allowed to stand for 30 minutes. The samples and standard were read against the blank at 560nm.

Total cholesterol was calculated as:

$$\text{Cholesterol (mg/100ml)} = \frac{\text{A TEST}}{\text{A STANDARD}} \times 200$$

where A TEST = Absorbance of samples

A STANDARD = Absorbance of standard.

The samples were assayed in triplicates.

3.7 DETERMINATION OF HAEMATOCRIT

The packed cell volume (PCV) was determined by the microhaematocrit method as described by Dacie and Lewis (1975). Well-mixed anticoagulated whole blood was taken up into capillary haematocrit tubes until they are approximately 2/3 filled with blood (air bubbles were avoided). One end of the haematocrit tube was sealed in a hot flame and the tubes placed in the radial grooves of the centrifuge head exactly opposite each other, with the sealed end away from the centre of the centrifuge and centrifuged for 5 minutes. The haematocrit was read using the microhaematocrit tube reading device.

3.8 DETERMINATION OF HAEMOGLOBIN

The haemoglobin content of the samples was determined according to the method of Schalm, et al., (1975)

3.9 DIFFERENTIAL CELL COUNT

(a) Blood smear:

Wedged blood smear was made by manual method as described by Dacie and Lewis (1975). A drop of blood was carefully placed in the middle of a very clean glass slide approximately 1cm from the end using an applicator stick. The slide was placed on a flat table top with the drop of blood on the right. The two left edges of the slide were held with the thumb and index finger of the left hand and the spreader slide with the thumb on the edge of one side and the other four fingers of the right hand on the edge of the other side. The end of the spreader slide was placed slightly in front of the drop of blood on the other side with an approximate 25° angle between the two slides. The spreader slide was drawn back toward the drop of blood. As soon as the spreader slide came in contact with the drop of blood, the blood began to spread to the edges of the spreader slide still keeping the spreader slide at a 25° angle and the edge of the spreader slide firmly against the horizontal slide, the spreader slide was drawn rapidly over the entire length of the slide.

The blood smears were dried quickly by waving rapidly in the air.

(b) Staining

The smears were fixed in methanol for 5 minutes in a coplin jar. They were removed and allowed to dry for about 20 minutes.

The smears were then stained in Giemsa stain (Appendix I) for about 30 minutes. The stained smears were removed, washed 2-3 times in tap water and allowed to dry.

(c) **Examination of the Stained Blood Smear:**

The blood smear was examined using the low power (10x) objective and the portion where there was only slight over-lapping of red cells was chosen. A drop of oil was placed on the slide and carefully changed to the oil immersion objective (100x).

The differential cell count and examination of the white cell morphology was performed beginning from the thin area of the slide where the red cells are slightly overlapping. The slide was gradually moved toward the base of the smear and each white cell seen counted and recorded on a differential cell counter until 100 white cells have been counted.

3.10 HISTOPATHOLOGICAL EXAMINATION

All palpable masses present in the mammary glands (in females) at necropsy, liver and scrotal sac (in males) samples were removed, fixed in 10% neutral buffered formalin (Appendix 2) and dispatched to the histology laboratory of Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for embedding, sectioning and staining. Histopathologic examination was conducted on all specimens.

3.11 STATISTICAL ANALYSIS

The data collected in the experiment were subjected to the student t-test at 95% level of significance. The weights of the animals were analyzed using the test statistic Z (P in both direction). Both statistical analysis were performed as described by Armitage (1971).

CHAPTER FOUR

RESULTS AND DISCUSSION

A total of 47 and 30 palpable growths were observed in groups A and B respectively. When expressed as number of palpable growth per rat however, no difference was observed between the two groups (Table 1). No difference was observed on the average number of palpable growth per rat on a weekly basis (Figure 2). The two oils did not significantly affect the latency period in both treatments (Table 1). However, subsequent animal growth was affected (though not significantly) as indicated by the initial body weight at the time of DMBA administration and the final body weight upon termination of the experiment (Table 1). Three rats in group A died during the 4th, 13th and 16th weeks respectively without a palpable growth and were not included in the analysis. Two other rats in the same group died in the 25th and 32nd weeks respectively, with palpable growths and these were treated as censored observation (Figure 2).

During the course of the experiment, female rats intubated with DMBA in sesame seed oil gained on the average 106 $\pm$ 21.2g while those intubated with DMBA in soyabean oil gained on the averge 126 $\pm$ 10.8g. There was no significant ($p > 0.05$) difference in body weight gain between the females in both treatments though the soyabean oil group gained on the average 19g more than the sesame seed oil group.

A near linear relationship between weight gain and age from week 0 to week 12 (Figure 3) was observed between the two treatment groups, while the relationship from week 14 to week 42 when the experiment was terminated was non-linear.

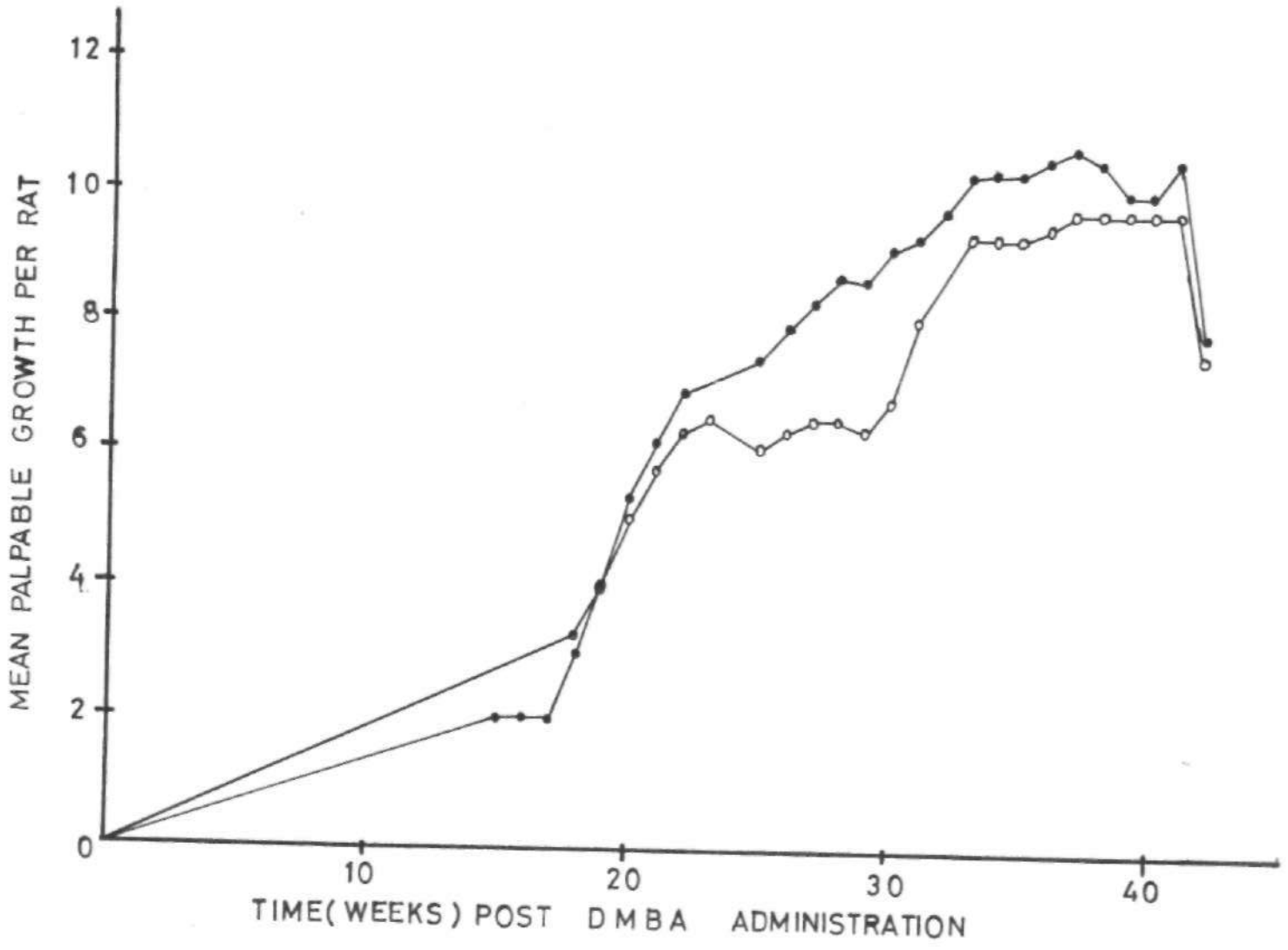
Table 1: Effects of sesame seed oil and soyabean oil as suspension media on the incidence and yield of Palpable growth in rats given a single gastric intubation of DMBA.

Treatment	Total No. of Palpable growths	Number of palpable growth/rat.	Latency period (Weeks)a	Initial Body Weight(g)	Final Body Weight(g)
Group A. DMBA in Sesame Seed Oil (n=6)	47	7.88 ±0.90	18.88 ±1.54	80.0 ±12.40	187.0 ±21.10
Group B. DMBA in Soyabean Oil (n=4)	30	7.50 ±1.12	18.50 ±0.50	80.0 ±13.60	206.0 ±10.80

Values are expressed as mean ± SD.

No significant difference between the two groups in the yield of palpable growth (P>0.05).

a. Time between DMBA administration and the appearance of the first palpable growth.



FIG·2: MEAN PALPABLE GROWTH PER RAT AT WEEKLY INTERVALS FOLLOWING DMBA ADMINISTRATION(WEEK 0)· THE RATS WERE INTUBATED WITH DMBA IN EITHER SESAME SEED OIL (●) OR SOYABEAN OIL (o)

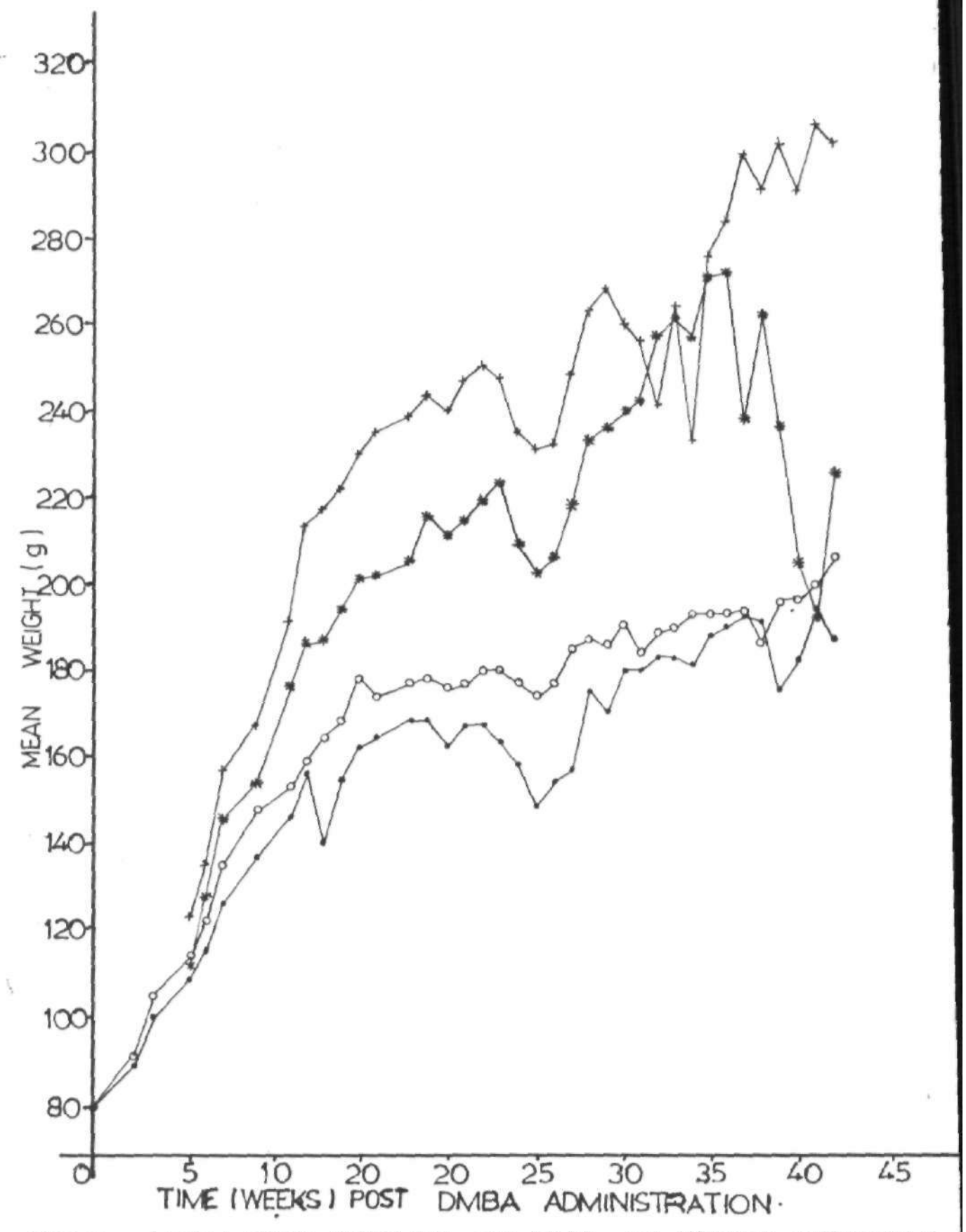


FIG.3: MEAN BODY WEIGHTS OF RATS AT WEEKLY INTERVALS FOLLOWING DMBA ADMINISTRATION (WEEK 0). THE RATS WERE INTUBATED WITH DMBA IN SESAME SEED OIL, FEMALES(•), MALES(+) AND DMBA IN SOYA BEAN OIL FEMALES(o), MALES(*).

The liver weight of female rats intubated with DMBA in soyabean oil was significantly ($P<0.05$) higher than that of female rats intubated with DMBA in sesame seed oil (Table 2), while no significant ($P>0.05$) variation in mammary gland weight was observed between the two groups.

Blood cell analysis of female rats taken when they were necropsied is shown in Table 3. There was no significant ($P>0.05$) difference in the haematocrit, haemoglobin levels and white cell differential counts in both treatment groups.

A significant ($p < 0.05$) difference was however evident in total serum protein and cholesterol levels for both treatments (Table 4).

For histopathological reports, the mammary glands were screened for lymph node infiltration by eosinophils, fibroblastic proliferation between the glands and hyperplastic adenoma. The liver samples were observed for any signs of congestion of central veins and sinusoids and for any gross pathological changes. Two rats in the soyabean oil group were observed to have mammary gland adenoma while one rat exhibited changes characteristic of fibroblastic proliferation between normal glands (Table 5). Three female rats showed marked congestion of some central veins and sinusoids of the liver and infiltration of the lymph nodes with eosinophils in the sesame seed oil group (Table 5).

The results in the present study demonstrate that rats fed DMBA in vegetable oils (sesame seed oil and soyabean oil) during the initial phase of DMBA induced mammary carcinogenesis display similar tumour

Table 2: Livers and mammary gland weights of albino rats intubated with DMBA suspended in two oils 42 weeks after DMBA administration.

	Final rat Body wt. (g)	Weight of Mammary Gland (g)	Mammary gland wt/ Unit Body Weight (mg/g)	Weight of Liver (g)	Liver wt/ Unit Body Weight (mg/g)
GROUP A. DMBA IN SESAME SEED OIL (n=6)	87.0 ±21.10	3.19 ±0.87	1.70 ±0.37	5.93a ±0.89	3.18 ±0.31
GROUP B. DMBA IN SOYABEAN OIL (n=4)	206 ±10.6	3.75 ±0.60	1.84 ±0.38	7.27a ±1.01	3.56 ±0.59
CONTROL (n=4)	172 ±4.30	3.02 ±0.17	1.70 ±0.05	4.82 ±0.19	2.80 ±0.06

Values are expressed as Mean ± SD.
a = $p < 0.05$

Table 3: Blood count of rats 42 weeks after DMBA administration.

TREATMENT	HAEMATOCRIT %	HAEMOGLOBIN %	NEUTROPHILS %	LYMPHOCYTES %	EOSINOPHILS %	BASOPHILS %	MONOCYTES %	BANDS %
GROUP A,								
DMBA in Sesame Seed Oil	32,75	10,79	40,67	52,67	2,00	1,67	1,33	1,67
(N=6) Females	±5,80	±1,78	±11,90	±10,52	±0,82	±2,36	±1,25	±2,36
GROUP B,								
DMBA in Soyabean Oil	34,00	11,34	50,67	41,67	1,67	0,67	3,00	2,33
(n=4) Females	±5,59	±1,87	±4,99	±5,91	±0,94	±0,94	±1,63	±1,25
CONTROL FEMALES	44,33	14,78	56,75	29,25	0,75	0,25	1,00	2,00
(n=4)	±1,63	±0,54	±5,26	±5,63	±0,43	±0,43	±0,71	±2,00
GROUP C,								
DMBA in Soyabean Oil	28,29 a	9,46 b	42,00	52,00	1,33	-	2,33	2,33
(n=7) Males	±2,05	±0,71	±7,79	±12,75	±0,47		±1,89	±2,63
GROUP D,								
DMBA in Sesame Seed Oil	39,50 a	13,16 b	35,00	61,50	1,00	0,50	2,00	-
(n=2) Males	±1,80	±0,61	±10,00	±9,50	±1,00	±0,50	±1,00	
CONTROL MALES	45,14	15,05	66,00	30,67	0,67	-	1,67	1,00
(n=6)	±1,81	±0,60	±5,35	±4,78	±0,47		±0,47	±0,82

Values are expressed as Mean ± S.D.

a = P<0,05
b = P<0,05

Table 4: Biochemical profile of serum samples from rats intubated with DMBA suspended in two types of oils.

Treatment	Total serum Protein (g/L)	Total Serum Cholesterol (mg/dL)
GROUP A.		
DMBA IN SESAME SEED OIL	83.3 a	50.5 c
(n=6) FEMALES	±4.48	±11.01
GROUP B.		
DMBA IN SOYABEAN OIL	117.0 a	128.7 c
(n=4) FEMALES	±33.11	27.38
FEMALES CONTROL	179.8	123.8
(n=4)	±3.47	±2.72
GROUP C.		
DMBA IN SOYABEAN OIL	79.3 b	65.8 d
(n=7) MALES	±6.19	±13.44
GROUP D.		
DMBA IN SESAME SEED OIL	59.2 b	44.1 d
(n=2) MALES	±6.19	±3.25
MALES CONTROL	107.7	75.4
(n=4)	±3.14	±6.96

Values are expressed as mean ± S.D.

a = $p < 0.05$
b = $p < 0.05$
c = $p < 0.05$
d = $p < 0.05$

Table 5: Result of histopathological examination of mammary glands and livers of treated animals taken when rats were necrosied.

TREATMENT	MAMMARY GLAND			LIVER	
	LYMPH NODE INFILTRATED WITH EOSINOPHILS	FIBROBLASTIC PROLIFERATION BETWEEN NORMAL GLANDS	HYPERPLASTIC ADENOMA	CONGESTION OF SOME CENTRAL VIENS AND SINUSOIDS	NO. GROSS PATHOLOGICAL CHANGE
GROUP A. DMBA in Sesame Seed Oil (n=6) Females	3	-	-	3	3
GROUP B. DMBA in Soyabean Oil (n=4) Females	-	1	2	-	1

Figures represent number of rats

development. Studies have shown that sesame seed oil (Eskin et al., 1968; Huggins, 1979; Elegbede et al., 1984, 1986a; Elson et al., 1988) olive oil (Alam et al., 1984) and corn oil (Sundram et al., 1989) are good media for preparing DMBA suspension for experimental DMBA-induced carcinogenesis. The latency period (that is. the time between DMBA administration and the appearance of the first palpable growth) in both sesame seed oil and soyabean oil treatments is also consistent with previous findings (Eskin et al., 1968; Huggins, 1979).

Both sesame seed oil and soyabean oil have similar composition (Table 6). They both contain about 12% saturated fatty acids of which palmitic acid constitutes 8% The unsaturated fatty acids of sesame seed oil are made up of about 45% oleic, 41% linoleic and 1% arachidonic acids, whereas the unsaturated fatty acids of soyabean oil consist of about 28% oleic, 54% linoleic and 5% linolenic acids. The similarities in composition, may account for the similar tumour incidence and response observed in the present study. Laboratory rats are very useful model for the investigation of susceptibility to breast cancer (both spontaneous and induced). However various strains and types of rats differ in their susceptibilities to mammary carcinogenesis with the Copenhagen rats being completely resistant (Gould et al., 1989; Moore et al., 1988).

This study confirmed earlier reports (Elegbede and Omage, 1989). that locally available strains of rats may be susceptible to mammary carcinogenesis and can thus be utilized in carcinogenic studies.

Table 6: Fatty acid composition of soyabean and sesame seed oils.

FATTY ACID	SESAME SEED OIL	SOYABEAN OIL
Myristic (C14:0)	0(0)	0 - 1 (0)
Palmitic (C16:0)	7-8(8)	7 - 11(8)
Stearic (C18:0)	4-5(4)	2 - 6 (4)
Oleic (C18:1)	37-49(45)	25-33(28)
Linoleic (C18:2)	35-47(41)	43-56(54)
Linolenic (C18:3)	0(0)	2-10(5)
Arachidonic (C20:4)	0-1 (1)	0-2(0)
Others*	-	(0.6)
Saturates*	13	12
Monounsaturates*	45	28.6
Polyunsaturates*	41	59

Values are expressed as percent (%) rate.

Source: Extracted from Cornelius, J.A. (1980). Rice bran oil for edible purposes, a review. Tropical Science 22(1): 1-26.

* Not included in the original table.

Figures in parenthesis represent the average of each range.

The growth of the female rats treated with DMBA in either sesame seed oil or soyabean oil was comparable throughout the experimental period (Figure 3). This indicates that there was no significant difference in energy intake and utilization among the two treatment groups. Sylvester et al. (1986) and Sundram et al. (1989) made similar observations.

The observed differences in the weights of the livers in both treatment groups may be due to the relative differences in the final rat body weights as indicated by similar organ weight per unit body weights of the animals (Table 2). Control animals with final weight close to either of the treatment groups gave similar liver weights (Table 2). Data from the present study do not produce evidence for an alteration in specific organ development by DMBA administration.

Haematological profiles of all treated animals shows that the haematocrit and haemoglobin levels were relatively low compared with those of control animals (Table 3). The elevated levels of lymphocytes in all treatment groups compared to control (Table 3) is expected since these cells play an important part in the build up of humoral and cellular immunities (Garovoy and Capenter, 1980). This elevated lymphocytes is an indication of immune response towards the carcinogen insult (Fefer et al., 1968).

The total serum protein and the total serum cholesterol were 41% and 156% respectively higher in the soyabean oil group than the sesame seed oil group. This elevated biochemical profile in the soyabean oil group requires close scrutiny. It is now established that saturated acids (but most probably not stearic acid) increase the plasma cholesterol content, whereas polyunsaturated fatty acids have a cholesterol-lowering

effect (Anon, 1987). The failure of sesame seed oil to elevate serum cholesterol as much as the soyabean oil may be due to its higher oleic acid content, its lower linoleic acid content or to the presence of a minor constituent. Both oils have similar composition of saturated and unsaturated fatty acids, however they were only applied once at intubation. The composition (Table 6) of the saturated fatty acids or other minor constituent in either of the oils may account for this anomaly.

The drop in the average palpable growth observed between weeks 41 and 42 (Figure 1) may be due to regression of the tumours (Young and Cohen, 1963). It is now established that both thymus-dependent, cell-mediated immune mechanisms (Fefer et al., 1968) and humoral antibodies (Allison et al., 1974; Gorden et al., 1982) play important roles in tumour regression.

The observed hyperplasia, disorganized and piling up of epithelial cells of some of the glands in two rats in the soyabean oil group indicate the presence of mammary gland adenoma. Though no such diagnosis were made in the other rats in this same group (except that one rat showed fibroblastic proliferation between normal glands) or in the sesame seed oil group (except that the lymph nodes in three rats were unfiltrated with eosinophils).

Over the period of this study, male rats intubated with DMBA suspended in sesame seed oil gained on the average 223 ± 14.1g while the soyabean oil group gained 146 ± 49.0g (Figure 2). There was significant ($P = 0.021$) difference in the body weight gain between the males in both treatment groups where the sesame seed oil group gained on the average 77g more than the soyabean oil group.

A near linear relationship between weight gain and age from week 0 to week 12 (Figure 3) was observed in the males while the relationship from week 14 to week 42 when the experiment was terminated was non-linear.

Male rats intubated with DMBA suspended in sesame seed oil had an average liver weight of 11.63 ± 0.780g while the soyabean oil group had 8.81 ± 1.161g (Table 7). There was a significant ($P < 0.05$) difference between the two groups. There was significant ($P < 0.05$) difference in the average weight of the scrotal sacs (5.78 and 8.54g) of the male rats in both treatment groups (Table 7).

The result of analysis of blood taken from male rats at necropsy is shown in Table 3. A significant ($P < 0.05$) difference was observed in both haematocrit and haemoglobin levels between both treatment groups. There was no significant ($P > 0.05$) difference in the white cell differential counts in both treatment groups.

A significant ($P < 0.05$) difference was also observed in the total serum proteins and total serum cholesterol in the males of both treatment groups (Table 4).

Table 7: Liver and scrotal sac weights of albino rats intubated with DMBA suspended in two oils 42 weeks after DMBA administration.

TREATMENT	Final Rat Body weight (g)	Length of Scrotal sac (cm)	Scrotal sac Length/Unit Body weight (cm/g)	Weight of Scrotal sac (g)	Scrotal sac weight/unit Body weight (mg/g)	Weight of Liver (g)	Liver wt/ Unit Body Weight (mg/g)
GROUP C.							
DMBA in Soyabean Oil	226	4,37	0,02	5,78 a	2,55	8,81 b	4,05
(n=7)	±49,0	±0,45	±0,00	±0,91	±0,48	±1,16	±0,72
GROUP D.							
DMBA in Sesame seed Oil	302	4,75	0,02	8,54 a	2,83	11,63 b	3,87
(n=2)	±14,10	±0,25	±0,00	±0,19	±0,07	±0,78	±0,44
CONTROL	228	3,88	0,01	6,38	2,22	10,95	3,81
(n=4)	±22,60	±0,22	±0,00	±0,76	±0,24	±1,84	±0,62

Value are expressed as Mean ± S.D.

a = P<0,05

b = P<0,05

In the males, the sesame seed oil group showed a higher average final body weight than the soyabean oil group. Similar growth pattern was expected since laboratory chow was provided ad libitum, and the animals were exposed to similar experimental conditions that might influence their rate of metabolism. Other factors that need further investigation (for example. the available floor space in the two cages) may be responsible for this difference.

The observed differences in the weights of the livers and scrotal sacs in the males in both treatment groups may be due to the relative differences in the final rat body weights as indicated by similar organ weight per unit body weight of the animals (Table 7). Control animals with final body weight close to either treatment groups gave similar livers and scrotal sac weight (Table 7). Data from the present study does not produce evidence from an alteration in organ development by DMBA administration.

The use of both male and female albino rats for each treatment serves to eliminate the possibility of any peculiar phyeiological response to the carcinogen/oils that may be elicited due to sex variation. There was significant ($P < 0.05$) difference in body weight gain between the males and females in each treatment (Figure 3). This difference may be due to sex variation. Biochemical analysis particularly the total serum proteins and total serum cholesterol and haematological studies particularly the lymphocyte counts are important in chemical carcinogenesis. The data obtained from this study shows

that in both male and female rats in each treatment, the total serum proteins, total serum cholesterol and lymphocytes gave a consistent pattern. This observation eliminates any sex variation in response to the carcinogen and the oils used in preparing the suspensions.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

From the results obtained in this study, there is indication that it is possible to substitute soyabean oil for sesame seed oil as DMBA solvent and the use of locally-available strain of albino rats as a model for experimental mammary carcinogenesis. The possibility of DMBA induced scrotal development is also indicated.

Biochemical profiles shows higher levels of total serum protein and total serum cholesterol in the females of the soyabean oil group than the sesame seed oil group. The liver weight was also higher in the soyabean oil group than the sesame seed oil group. Similar trend was observed in the males where higher levels of total serum protein, total serum cholesterol was observed in the soyabean oil group than the sesame seed oil group. However higher scrotal sac weights were observed in the sesame seed oil group than the soyabean oil group.

Investigations of environmental xenobiotics that may play a role in the aetiology of organ specific human neoplasms or dietary inhibitors of carcinogenesis have mainly relied on either animal testing or the study of the interactions of the xenobiotics or inhibitors with non-target mammalian cells in vitro. Prediction of carcinogenicity for a given chemical is a major concern for government, the chemical industry and the public. A basic problem in experimental carcinogenesis, apart from the susceptibilities of locally available strain of rat, is the relatively high experimental costs, particularly the maintenance of animals (due essentially to scarcity and high costs of commercial laboratory animal chow). A locally-formulated feed (ABU Pharm. Feed)

has been shown to be more cost-effective than the commercial feed and is also of comparable quality (Kwanashie et al., 1989). This feed is recommended for use in experimental carcinogenesis.

The results obtained in this research requires further investigation. Also other locally available oils such as groundnut oil, sunflower seed oil and palm oil should be analyzed for their potential properties as DMBA solvent and their effects on serum protein and serum cholesterol and plasma lipoprotein profiles.

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APPENDIX I

PROTOCOL FOR ORAL INTUBATION WITH DMBA

A. Carcinogen preparation

The weight of the rats were taken so as to know the amount of carcinogen to weigh out. Scale warming, DMBA warming were started.

Dosage: 65mg DMBA/1000g weight of rat

or 0.065mg DMBA/g of rat.

$$\text{\# rats:} \quad \frac{\text{x avg. gram weight of rat + 20\% extra}}{\text{total weight of all rats.}}$$

$$= \quad \frac{\text{x. 0.065mg/g rat}}{\text{total \# mg DMBA needed for experiment}}$$

$$\frac{\text{total \# mg DMBA}}{\text{6.5mg/ml sesame oil/soyabean oil.}}$$

= total oil to mix with DMBA

1. Clean dry glass beads were used.

2. Older glass 50ml Erlenmeyer (1.5ml of glass beads were placed in each flask) were used.

3. The required quantity of oil (13mls) was measured into each flask. The required quantity of carcinogen (84.5mg) was added into each flask. Foiled cap was then placed on the flasks which were then foiled to the neck. A second cap was placed over the top and taped. Each flask was labelled either DMBA in sesame oil or DMBA in soyabean oil.

4. A last layer of aluminium foil was placed with uncontaminated (gloved) hands so that the outer foil remains carcinogen free.

5. A water shaker bath which had been allowed to heat up to 100°C (this was done earlier while making up carcinogen) was set up to incubate carcinogen.

6. Carcinogen were allowed to shake for 1 hr at 90°C.

7. Carcinogen were allowed to cool down to body temperature before intubation in order not to scald rats.

The amount of carcinogen to feed each rat was calculated thus:

0.01ml oil/g of rat is needed

1ml/100g of rat is also needed

also 6.5mg DMBA/100g of rat is needed. So, 6.5mg DMBA/ml of solution was made above.

To find the mls of solution to feed each rat:

8. $\frac{\text{Weight of rat}}{\text{100g of rat/ml}}$ = # mls of 6.5mg/ml carcinogen to give to the rat.

9. Since each rat weighed approximately 80g.

$$\frac{80\text{g}}{\text{100g of rat/ml}} = .8\text{mls of 6.5mg/ml DMBA}$$

Precaution:

1. Gloved hands (3 layers of gloves) were used throughout the preparation.

2. Nose and mouth mask was also used.

3. Contact between the carcinogen and the naked skin was avoided.

B. PROTOCOL FOR PALPATION

1. The rat was held by the skin of the neck and turned to reveal the ventral view.

2. The distribution of the nipples were determined by rubbing the hand over the ventral surface of the abdomen.

3. There are usually six pairs of teats : 3 thoracic, one abdominal and two inguinal (Plate 2).

4. Clean pebbles of known volumes (or diameters) were made handy.

5. Palpation involves, feeling each nipple i.e. thoracic nipples, axillary nipples, abdominal nipples, and inguinal nipples between the thumb and the second finger for palpable growth.

6. Where a palpable growth was observed its volume (obtained by comparing it with the known volumes of the pebbles) and location was recorded.

7. Subsequent increase in the volume of palpable growths and new growths were recorded.

Plate 3: Ventral view of a mature female rat



Protocol for palpation: There are usually 6 pairs of teats: 3 thoracic, 1 abdominal and 2 inguinal.

1. Thoracic nipples
2. Axillary nipples
3. Abdominal nipples
4. Inguinal nipples
5. Clitoris

APPENDIX II

PREPARATION OF REAGENTS

A. 10% Neutral-buffered Formalin

Sodium dihydrogen phosphate (anhydrous)	3.5g
Disodium hydrogen phosphate (anhydrous)	6.5g
Concentrated formaldehyde solution (40%)	100ml
Distilled water	900ml

The formaldehyde solution was combined with the water and the two salts were added making sure that the first salt dissolved before the second was added. Tissues were placed in 25 times their volume of the buffered formalin solution as soon as possible.

B. Giemsa stain

Giemsa powder	3.8g
Glycerol pure	250.00ml
Methyl alcohol (Analar)	250.00ml

The measured quantity of dry dye was added directly to a hard glass (Pyrex) bottle containing alcohol-glycerol mixture and at least 50 clean glass beads of about 5mm in diameter. The bottle was tightly stoppered immediately to keep out any moisture. The dye was allowed to sink for some minutes and the bottle was shaken thoroughly for about 5 minutes and left on the laboratory bench. The shaking process was repeated 6-10 times at half-hourly intervals a day for a minimum of 3 days. The stain was preserved in the refrigerator.