

SOME ANTHELMINTIC AND OTHER PHARMACOLOGICAL ACTIONS OF
DIETHYL ETHER EXTRACT OF THE SEEDS OF
CARICA PAPAYA LINN

BY

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A

THESIS

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(i)

DECLARATION

I hereby certify that the work reported in this thesis was carried out by me under the supervision of Dr. A. K. Absitey and Dr. O.C. Wambebe in the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences. The work of other investigators is acknowledged and referred to accordingly. I declare that no part of this thesis has been submitted elsewhere for a degree.


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CERTIFICATION

This thesis entitled, "Some Anthelmintic And Other Pharmacological Actions Of Diethyl Ether Extract Of The Seeds Of Carica papaya Linn" by Billeya James Paninga meets the regulations governing the award of the degree of MASTER OF SCIENCE (Pharmacology) of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To those who have died in Africa due to
helminthiasis.

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ABSTRACT

The anthelmintic property of the extract of Carical Papaya seeds against Nippostrongylus braziliensis and Hymenolepls nana were studied using rats. In addition, pharmacological properties of the seed extract on skeletal, smooth and cardiac muscles were investigated. The extract (0.5 - 2 g/kg) used once daily for three days, or once followed by purging with magnesium sulphate (4.6 g/kg) 2 and 2k hours later, did not show any activity against N. braziliensis. The activity of bephenium hydroxynapthoate (150 - 200 mg/kg) against N. braziliensis. was significant.

On the other hand, the extract exhibited activity both in vitro and in vivo against H. nana. The LD₅₀ for extract and niclosamide in vitro were 36 ug/ml and 120 ng/ml respectively. The extract at concentrations above 100 ug/ml either paralysed the worms or killed them. A similar effect was observed with niclosamide (>1.9 ug/ml).

The activity of extract was significant at concentrations above 0.5 g/kg when used once daily for three days against H. nana. When used once, followed by purging with magnesium sulphate, it was significant at 1.0 g/kg and highly significant at 2.0 g/kg. Niclosamide (600 mg/kg) once daily for three days showed complete deparasitization of all

the rats used. The results of the extract suggest that it could be a cestocidal agent but its use as a general vermifuge is not justified in ankylostomiasis.

The extract (> 32 ug/ml) affects isolated skeletal muscle preparations directly resulting in permanent contracture, suggesting that it could be acting at a point beyond the neuromuscular junction. It is likely to be interfering with the process involved in, either sequestering of released Ca^{2+} or the storage of Ca^{2+} thereby making Ca continuously available for sustained and irreversible contracture.

The extract (0.5 mg/ml) contracted and desensitized both oestrous and non-oestrous rat uterine smooth muscle to oxytocin and acetylcholine. The activity may be related to a possible effect of the extract on the fluxes of calcium or other ions.

On the cardiac muscle, the extract (0.2 mg/ml) produced initial positive chronotropic and inotropic effects followed by gradual decrease in force and rate of contraction of the heart. It also desensitized the heart to acetylcholine and adrenaline. The cardiac effect of the extract may be due to its interference with enzyme systems (e.g. c-AMP, c-GMP) responsible for the mobilization of Ca^{2+} .

When acute toxicological studies with chicks and mice were conducted, the extract (2 g/kg) did

not produce any observable toxic effects.

BITC was qualitatively determined by its hydrolysis to yield hydrogen sulphide and carbon dioxide gases. It is therefore likely that the observed effects of the extract in this project may be principally attributed to BITC content of the seed extract.

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CHAPTER I

INTRODUCTION

1.1 Helminth

"Helminth" is derived from the Greek words "helmins" or "helminthos", a worm. It is strictly applied to three phyla of parasitic worms, namely

- (a) Platyelminthes or flat worms which include Trematodes (flukes) and Cestodes (tapeworms),
- (b) Nemathelminthes which include all round worms and
- (c) Annelids which include earthworms, leeches and other ringed worms (Nnochiri, 1975).

Helminths as parasites of man and animals have been known since the early times. According to Stoll (1937), more than 800 million people of the world are affected by helminthiasis. The incidence is more in the Tropics due to climatic and sociological factors (Cavier, 1973).

Although helminths do not account for as many deaths as the protozoan parasites of man, they probably cause more morbidity and more economic and social deprivation among the population affected than any single group of parasites (Nnochiri, 1975).

The economic loss due to helminthiasis in ruminants alone in Nigeria has been estimated to be at least 144 million naira annually, resulting from death, weight loss and liver damage (Akerejola,

van Veen and Njoku, 1979). The major constraint in combating helminthiasis in Nigeria is unavailability of drugs which is related to socio-economic and political reasons.

The use of medicinal plants to combat helminthiasis in both man and animals has been recognized long ago. The Fulani herdsmen in Nigeria realize animal helminthiasis to be a problem and more significant in calves of less than a year old, as a result, herbal treatment is commenced within one week of birth as a routine (Ibrahim, Nwude, Aliu and Ogunsisi, 1983).

Nigeria, and indeed the continent of Africa as a whole, is blessed with both plants of tropical and temperate types. Despite the fact that some studies have been conducted on medicinal properties of some of these plants, the continent is still under-explored (Puri and Talalaj, 1964). Some of the plants used traditionally as anthelmintics in Africa that have been studied include Hunteria umbellata which was said to be equipotent to piperazine base in the management of ascariasis (Onuaguluchi, 1964), Allium sativum used in Nigeria as vermifuge was shown to have some activity against Ascaridia galli in chicken (Das and Thakuria, 1974), Diospyrol from the plant Diospyros mollis was shown to be superior to bephenium against Necator americanus in hamster and against Hymenolepis nana in mice (Sen, Joshi, Parthasarathy, Kamat, 1974).

Among other plants which are common in Nigeria, and whose anthelmintic properties have been reported is Carica papaya L.

1.2 Carica papaya Linn

1.2.1 Origin

Carica papaya Linn, popularly known as pawpaw or papaw belongs to the family Caricaceae which consists of the following genera; Carica, Cyclicomorpha, Jacaratia and Javilla (Storey, 1969). Carica which contains about twenty one species is the only genus that is cultivated for its fruits.

It had been earlier thought that the plant is indigenous to both Africa and Asia. However, de Candolle (1964) maintained that the plant is of American origin. The argument put forward by de Candolle (1964) was that the plant is easily naturalized outside plantations hence could easily adapt to Africa and Asia. He contended that in modern Indian languages, it bears names derived from the American word 'papaya'.

It is now believed by botanists that Carica papaya originated from the lowlands of Central America, somewhere in the region of Southern Mexico and Nicaragua. Following the discovery of America, it was distributed along tropical trade routes by Spanish and Portuguese sailors. It reached Panama as early as 1835, Puerto Rico by 1540, Cuba soon

thereafter, By 1611, it was grown extensively throughout the tropical and extratropical regions of the world, both as plantation tree and as favourite fruit for home garden (Storey, 1969; Chandler, 1964). The plant has importance as plantation crop in Hawaii (Solo and Bush varieties), South Africa (Hortus Gold variety), Australia (Improved Petersen variety), Florida (Betty variety), India, Sri-Lanka, the Philippines and a number of countries in Tropical America and Southeast Asia.

1.2.2

The Plant and its Economic Values

The plant is barely a tree, almost herbaceous but the trunk is persistent sometimes for a considerable number of years. The plant could be branched, but mostly unbranched with a head of gigantic, deeply lobed leaves with long stalks. Its very large leaves contribute to its tree-like appearance. It may be as tall as fourteen metres or higher with a stem of 30 cm in diameter, tapering gradually to about 10 - 13 cm at the summit and composed of soft spongy wood, mostly hollow in the centre (Linley and Moore, 1876).

Carica papaya is a polygamous species of plant. The trees, according to Storey (1969) are classified into three primary sexes namely; staminate or male, hermaphrodite or bisexual and pistillate or female.

Male plants bear large pendent panicles of yellow flowers. Hermaphrodite trees have short

inflorescences usually consisting of only five or six flowers that are exclusively pistillate. Pollination is effected mainly by wind but insects, especially moths, are believed to play a part also.

An important characteristic of Carica papaya is the presence of near the surface of stem, leaves and fruit of latex tubes filled with milky juice that contain the enzyme papain.

Depending on the variety, the mature fruits of female trees may be nearly spherical to slightly oblong and weighing between 0.4 - 2 kg or more. The Solo variety of Hawaii are pyriform and weigh between 0.4 - 0.5, Hortus Gold variety of South Africa are globular and weigh 1.25 - 2.5 kg. In many countries in Latin America and South Pacific Islands, large size seems to be considered an attribute of desirability and weights of fruits may be from 2.5 - 6.0 kg. In Nigerian markets (Jos and Zaria), the fruits (oblong to globular in shape), weigh 0.5 - 3 kg. The central cavity of the ripe fruit is surrounded by a host of small black seeds, covered by a jelly produced by the seed coat. There are modern varieties which are seedless.

The flesh of the ripe fruit is orange in colour and has a sweet musky flavour when eaten raw. The water content is from 85 to 88% or higher. Carbohydrates, mainly reducing sugars, vary from 7 to 12% while the protein content is about 0.5%.

Fat and Starch are not detectable. There is a lot of carotene in the flesh amounting to 2500 I.U. per 100 g of the flesh. The ascorbic acid content is also high and varies from 30 to 120 mg per 100 g of the fruit. There are also 20 mg of calcium, 0.5 mg of iron and 0.03 mg of thiamine per 100 g of the flesh (Nelson, 1951).

The volatile components of Carica papaya fruits have been analysed by some workers (Katague, 1964; Katague and Kirch, 1965). In these studies, homologous series of normal primary alcohol from C₁ to C₆ and primary isoalcohols from C₃ to C₅ were reportedly found, along with the corresponding acetate esters. Ethyl, propyl, butyl and hexyl alcohols and methyl, ethyl, amyl and isoamyl acetates were found to be present in relatively high concentrations (greater than 5%). Amyl and isobutyl alcohols and heptanone-2 were found to be present in quantities less than 1%. Also reportedly found were isopropyl alcohol, propyl acetate and butyl acetate. The concentration of these components varied at different stages of ripening. A decrease in the concentrations of methyl alcohol, methyl acetate, ethyl acetate and hexyl alcohol as the fruit ripens while a marked increase in the concentrations of ethanol and isoamyl acetate were noted.

In another work, Flath and Forrey (1977) concentrated the volatile components of the fresh

Carica papaya fruits and the concentrates were examined by combined gas chromatography - mass spectrometry (GC-MS) and a total of 106 components mainly alcohols, aldehydes and esters were identified. Linalool was said to be the major component of the concentrates followed by benzyl isothiocyanate.

Apart from being cultivated for the fruits as a source of food, Carica papaya plant is grown also for its latex, which contains four components with proteolytic activity, three of which have been identified as papain, chymopapain, and peptidase A. They represent 5, 27 and 14% of the latex protein respectively (Schack, 1967; Robinson, 1975). All parts of the plant contain this latex which can easily be seen exuding as a white sap when the plant is wounded. However, the greatest amount is in the green fruit where it occurs in the mesocarp of the ovary wall. After the fruit has reached maturity and begins to ripen the latex and enzymes hydrolyze into reducing sugars and possibly other substances and virtually none is found in the fully-ripened fruit (Storey, 1969). The amount of latex in a fruit has been found to be directly proportional to its size (Jones, Storey, Parri and Holdaway, 1940). The weights of the latex in all varieties and strains investigated by Jones et al. (1940) ranged from 0.7 - 1.0% of the weights of fruits, and dried latex weigh only 1% of the fresh latex.

1.3

Medicinal Uses

Different parts of Carica papaya have been used by different traditional healers for various ailments.

The roots of the male plants, according to Sofowora (1982) are used by traditional medical practitioners to cause immediate delivery. Puri and Talalaj (1964) reported that the practitioners use the roots for stomachache.

Dead leaves fallen off the trees are used in many decoctions for bathing children (Sofowora, 1982) and as arbotifacient (Puri and Talalaj, 1964). The fruits are used in gonorrhoea, and the seeds as vermifuge (Puri and Talalaj, 1964). Traditional healers in the Northern states of Nigeria use the seeds to expel worms. Robinson (1958), claimed that the fresh and dried seeds have been used in clinical trials and were found to be as effective as piperazine in the treatment of children suffering from ascariasis. It was also found to be effective in oxyridae in mice (Mehta and Parashar, 1966) and ascarides in dogs (Nagaty, Rifaat, and Morsy, 1959). Lal, Chandra, Raviprakash and Sabir (1976) confirmed that the fresh and dried seeds of Carica papaya were equipotent to piperazine in Ascaridia galli of birds.

1.4

Phytochemical and Pharmacological Studies

Phytochemical and pharmacological studies of

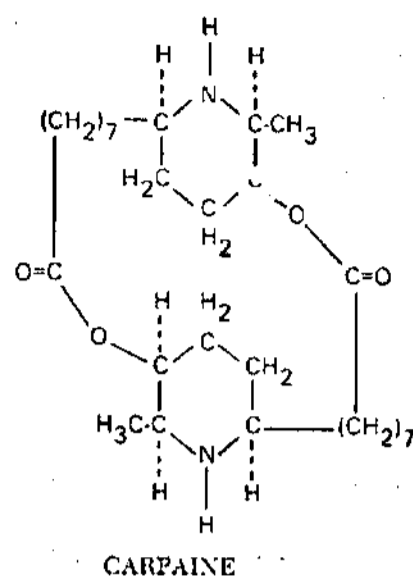
different parts of the plant have been reported in the literature.

1.4.1

The Leaves

Carpaine is the major alkaloid of Carica papaya and occurs in all the green parts of the plant (Burdick, 1971). It was first isolated in 1890 (Greshoff, 1890). Barger (1910), Barger, Robinson and Work (1937) extracted it from the leaves and tentatively established its structural formula. Later, pseudo-carpaine, which is described to be a diastereoisomeric with carpaine was isolated (Govindachari, Pai and Narasimhan, 1954). Carpaine occurs in Carica papaya leaves in concentrations as high as 0.4% and pure form is only slightly soluble in water, but its hydrochloride is readily soluble. It is a secondary amine, optically active being dextro-rotatory. It forms colourless lumps from ethanol, but soluble in chloroform and benzene. It has a sharp melting point of 121°C (Burdick, 1971).

Despite the fact that chemical identity of carpaine was known in the 1930s, it was only of late that the chemical structure was established (Spiteller-Friedman and Spiteller, 1964; Coke and Rice, 1965).



The pharmacological properties of carpaine have been reported in the literature. The Japanese have reported it to be effective in the treatment of amoebic dysentery (To and Kyu, 1934). Ramsawamy and Sirsi (1960) found carpaine to inhibit Mycobacterium tuberculosis at a low dilution of 10^{-4} .

On the cardiovascular system, carpaine was found to have a dose-dependent effect in lowering the blood pressure and heart rate of the rat (Hornick, Sanders and Lin, 1978) and the cat (Tuffley and Williams, 1951). The hypotensive and bradycardial effects of carpaine were not mediated through autonomic nervous system. Since selective autonomic nervous blockade with atropine sulphate (1 mg/kg) and propranolol hydrochloride (8 mg/kg) did not alter the

circulatory response to carpaine (Hornick et al., 1978).

These findings contradict the earlier speculations that carpaine may have pharmacological properties of digitalis (Fairchild, 1943) and the claims of Noble (1946) that doses of 20 mg of carpaine per day in humans acted very similar to digitalis.

Tuffley and Williams (1951) and Hornick et al. (1978) reported that carpaine affects the myocardium directly. Hornick and his colleagues (1978) suggested that the observed effects of carpaine on the myocardium may be related to its macrocyclic dilactone structure, a possible chelating structure. If the free calcium ions in the plasma were taken up by carpaine, a lowered cardiac performance would result.

Employing in vitro preparation, Tuffley and Williams (1951) found that carpaine produced a relaxation of guinea pig uterus, dilatation of guinea pig bronchioles, but the frog was relatively insensitive to the drug.

Carpaine was also found to be effective as an antitumour agent (Oliveros-Belardor et al., 1970).

Head and Lauter (1956) isolated, in addition to carpaine, small amounts of flavonols, tannins and organic acids from the leaves.

Papaya leaf-smoking is said to soothe the human upper respiratory tract instead of irritating it as tobacco smoke does (Finberg, 1960). This seems to be opposite to the effect of tobacco.

However, Smalberg, Rall and De Waal (1968) claimed to have extracted nicotine (0.0128%), cotinine (0.00278%) and a fourth, alkaloid that was not identified from Carica papaya leaves using alcoholic solvents.

In Nigeria, Ogan (1971) found the yield of carpaine from a local variety of Carica papaya in Ibadan to be 0.0115% of the dry weight of the leaves as against yields of up to 0.2% reported for Malayan plant or even 0.4% by Burdick (1971). The yield of carpaine according to Ogan (1971) remained uniformly low throughout the twelve successive monthly estimations and was not improved even by the use of younger leaves as suggested by Barger et al., (1937). Instead, the yield of choline was found to be up to 0.02% of the dried leaves. It was shown that the methanol extract containing the choline had oxytocic effect in vitro on guinea-pig uterus but not on rat uterus. Ogan (1971) suggested that the stimulant action of the methanol extract on the smooth muscle is related to the well known stimulant action of acetylcholine on smooth muscle. The significance of this, according to him as regards some of the alleged medicinal properties

of Carica papaya was uncertain, in view of the finding that rat uterine tissue (which usually approximate closely to human uterine tissue in response to oxytocic drugs) was unaffected.

1.4.2 The Bark and Root

There seems to be little work done on the roots and 'bark' of the plant or probably due to lack of activity or useful constituents the results were not reported.

However, the 'bark' has been used by traditional medicinal practitioners for the treatment of jaundice (Boum, Pousset, Lemonnier and Hadchoual, 1978). From the 'bark' of Carica papaya xylitol and some monosaccharides (mainly glucose and fructose) have been isolated and used on experimental jaundice in rats. It was found that there was significant drop in the level of unconjugated bilirubin (Pousset, Boum and Cavé, 1981).

Since it is possible to have bilirubin conjugate with monosaccharides in the process of getting rid of the bilirubin from the body, it is likely then that this pathway of conjugation, though not the main one is affected by xylitol and the monosaccharides from the 'bark'.

1.4.3 The Fruits

Papain, chymopapain and peptidase A are found in the latex obtained from Carica papaya plant, but occurs

mostly in the unripe green fruits. Papain is the most studied, though the crude papain is a combination of all these three components.

Papain has a molecular weight of about 23,000 and is made up of 198 amino acids in a single polypeptide chain with three disulphide bridges and an active sulphhydryl group. It has a wide enzymatic specificity against protein and low molecular weight substances (Smith, 1964).

Papain has been used commercially in both food and drug industries. The major use in the food industries is for tenderizing meat. It also used in making 'Chill-proof' beer, (i.e. traces of protein are digested at the end of brewing process to prevent turbidity on cooling) (Balls, 1941). Other uses include the tanning of skins and hides and previously, in the manufacture of chewing gum (Hill, 1937).

The medicinal use of papain includes its application for the treatment of insect stings (Arnold, 1972), jelly fish stings (Loder, 1973) and debridement of burn eschars and neurotic tissue (Shapira, Giladi and Neuman, 1973). Indeed, about 119 patent medicines have been listed as containing papain. Other effects of papain includes its analgesic and anti-inflammatory activities orally of which it was claimed that its potency was not significantly different from that of acetylsalicylic acid (Emelle, Shanaman

and Winbury, 1966). The Russians have used it both in animals and human beings to remove cataract (Starkov, Saprykin, Savinykh, 1970, Starkov and Savinykh 1971; Chutko, Ryshkina, Paulova and Yandiev, 1972).

Some unwanted effects or activities of papain have been reported. Papain acting as allergin has induced asthma to meat tenderizing workers who have had to be exposed to papain dust. Immunological studies in these patients have revealed the presence of specific Ig E antibodies (Novey, Marchioli, Sokol and Wells, 1979) and IgG (Tarlo, Shaikh Bell Cuff, Davies, Dolovich and Hargreave, 1978).

When papain is introduced into lungs of laboratory animals (rats, hamsters, rabbits, dogs) by either endotracheal instillation or aerosol inhalation, it rapidly produces emphysematous changes and is used as a model of human emphysema (Gross, Babyak, Tolker and Kaschak, 1964; Giles, Finkel and Leeds, 1970; Pushpakom, Hogg, Woolcock, Angus, Macklem and Thurlbeck, 1970; Johnson, Pierce and Reynolds 1971). However, there is no documentation of production of emphysema in man (Tarlo et al., 1978). Reports of pathology in man appear limited to allergic reactions of asthma, rhinitis, urticaria, angioedema, and anaphylaxis (Beecher 1931; Osgood, 1945; Milne and

Brand, 1945). In another work, itching which is common to all endopeptidases (Arthur and Shelley, 1955) was produced by papain without releasing histamine in human beings. The itch was not significantly altered by antihistamines. It had a more pricking character and did not produce vascular reactions (redness, weal and flare) which are seen when histamine acts on skin (Hägermark, 1973).

When administered orally or intraperitoneally to pregnant rats, papain has been shown to produce teratogenic and embryotoxic effects (Singh and Devi, 1978). In a similar experiment using rabbits, foetus exhibited stunting, visceral and subcutaneous haemorrhages and oedema of various organs as well as of body. The foetuses of rabbits that were allowed to deliver and grow were all dead within 1 to 2 weeks of birth and they showed gross histological changes (Devi and Singh, 1978).

The effects produced by papain on placentae of rats were haemorrhagic, cytolysis and degeneration (Devi and Singh, 1978). While the mechanisms of actions of papain on pregnancy have not been fully explained, attempts to digest chorion with many enzymes like trypsin, chymotrypsin, elastase, collagenase, ribonuclease, lysozyme and hyaluronidase have failed, but with papain, it has been possible to dissolve chorion (Kaighn, 1964).

The unripe fruits and seeds of Carica papaya have been used in some parts of India for criminal abortion (Modi, 1969) and pregnant women are strictly prohibited from eating Carica papaya fruits any time during pregnancy for fear of inducing abortion (Chopra, Chopra, Handa and Kapur 1958; Watt, 1972). Some tribes in India also believed that Carica papaya has powerful antifertility property (Gwatkin, 1964). In the study of Gopalakrishnan and Rajasekharasetty, (1978), they found that unripe fruit of Carica papaya induced abortions in rats. However, when stale and ripe fruits were used, the pregnancy terminating effect decreased by 50%.

1.4.4 The Seeds

The anthelmintic property of the seed of Carica papaya has been said to be vested in its volatile component. Dar, Garg and Pathak (1965), reported that the major components of the seed are carbasemine (Benzylthiurea) and benzyl isothiocyanate in aqueous extract after steam distillation and the anthelmintic property has been attributed principally to benzyl isothiocyanate (BITC).

The precursor of BITC is benzyl glucosinolate. The glucosinolates (thioglucosides) are a group of compounds distributed among the members of the plant families of Cruciferae, Moringaceae, Cappadaceae, Tropaeolaceae, Caricaceae, Grostemonaceae, and Salvadoraceae (Ettlinger and Kjaer, 1968).

BITC extracted from *Carica papaya* seeds was tested against 57 different microorganisms and was shown to be active against bacteria, yeast, and moulds. It is said to be fungicidal at relatively low concentrations (5-50 ug/ml) while bactericidal effect was observed in concentrations up to 100 ug/ml after contact for as long as four days, and gram-positive bacteria seemed more sensitive than gram-negative bacteria (El-Tayeb *et al.*, 1974).

The toxicological studies of BITC have been reported. At a dose of 30 mg/kg body weight in rats, there was no toxic symptoms except local irritation (Dar *et al.*, 1965). With doses as high as 200 mg/kg to 2.0 g/kg, there was no fatality but there was increase activity, excitation and ptosis in mice, indicating that the level of toxicity was low (El-Tayeb *et al.*, 1974).

The anthelmintic action of the extract was said to be due to its ability to block neuromuscular activity of the ascaris worms (Robinson, 1958; Krishnakumari and Majumder, 1960; Bose, Saifi Vijayavariga and Bhagwat, 1961). However, El-Tayeb and co-workers (1974) reported that the lack of effect of BITC on the rat phrenic nerve does not support the mechanism of action of most anthelmintics that block neuromuscular action of ascaris worms, hence may not effect its action through neuromuscular junction as other anthelmintics do.

1.5 Aims and Objectives of Present Study

The data reported in the literature shows that Carica papaya seed extract manifests bactericidal and fungicidal activities (El-Tayeb, 1974), anthelmintic activity in the family Ascaridae (mainly against ascaris in man, and Ascaridia galli of birds) and oxyridae in mice (Robinson, 1958; Mehta and Parasha 1966). There has been no report on the activity of the extract on Ancyslostomidae (Ancylostoma duodenale and Necator americanus).

In the literature, there are conflicting reports on the pharmacological action of the extract. El-Tayeb et al., (1974) reported that it has no activity on the rat phrenic-nerve diaphragm preparations. Other workers reported that the extract was able to block neuromuscular activity of the ascaris worms (Robinson, 1958; Krishnakumari and Majumder, 1960; Bose et al., 1961).

The objectives of this study therefore are:-

1. to investigate the anthelmintic property of the seed extract on other classes of worms, namely hookworms and tapeworms, Since the traditional practitioners use the extract as a general vermifuge.
2. to study the neuromuscular property of the seed extract in order to elucidate the possible mechanism of action of the extract at the neuromuscular junction.

CHAPTER IIMATERIALS AND METHODS2.1 Chemical and Drugs

The drugs and chemicals used in this project include the following:-

Magnesium sulphate (British Drug House)
Diethyl ether (May and Baker)
D-Tubocurarine chloride (Sigma Chemical Company)
Acetylcholine chloride (Sigma Chemical Company)
Adrenaline (Sigma Chemical Company)
Oxytocin (Pitchter)
Verapamil (Knoll, AG)
Niclosamide (S.A. Zurich-Milan) and
Bephenium hydroxynapthoate (Scanpharm).

2.1.1 Preparation of Drug Solutions

All appropriate drugs were weighed and dissolved in physiological saline except for the extract that was emulsified in 3% Tween 80 using a Hand-homogenizer (Mamido Dale Model, England). All solutions were prepared fresh for each day's experiment to avoid deterioration upon storage.

2.2 Animals

For anthelmintic experiments, albino rats, Wistar strain, were bred separately from those of the Animal House, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria. Animals were

given mebendazole 0.6 mg/kg in their drinking water for three days and the treatment repeated after two weeks. Deworming the animals was necessary so that the progenies will not have any worms thereby acquiring immunity. The progenies (6-8 weeks old) used weighed between 70 and 100 g.

In other experiments, albino mice, adult rats and guinea-pigs of either sex were obtained from the Animal House. The chicks used were obtained from Arewa Agricultural Enterprises Ltd., Zaria.

2.3 Plant Material

Ripe fruits of Carica papaya were purchased from the markets in Zaria and Jos in November 1984. The fruits were sliced open and the seeds removed and washed. During the process of washing, the jelly outer covers of the seeds were removed, after which the seeds were dried in the sun for two days. The dried seeds were packed and kept at room temperature for two weeks before extraction.

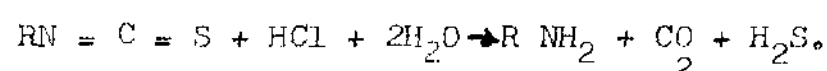
2.3.1 Extraction

The seeds were ground into fine particles in a mortar in batches of 200 gm and put into a conical flask of 1000 ml capacity. Warm water (100 ml) at 50°C was poured into the flask to moisten the powder and the flask immersed in a water bath at a temperature of 55°C for one hour, after which it was allowed to cool to room temperature. Diethyl ether (200 ml) added to the

flask and shaken gently for 20-30 min to allow for the extraction into the ether phase. Each 200 g of the seeds was extracted twice with 200 ml of diethyl ether. The liquid content of the flask comprising aqueous and ether components was removed and put into a separating funnel. The ether phase was separated and concentrated under vacuum using a rotatory evaporator (Rotavapor-RE, Büchi, Switzerland) and left in an oven (BS-Galenkamk) at a temperature of 45°C for 24 hours to get rid of any ether left behind. The extract obtained was weighed and stored in a freezer for pharmacological study.

2.3.2 Detection of Hydrogen Sulphide

Isothiocyanates upon boiling with acids (for example concentrated hydrochloric acid) are hydrolysed to primary amines and hydrogen sulphide is evolved.



When this reaction was carried out with extract, the gases evolved turned lime-water milky. A filter paper soaked in silver nitrate solution (0.1N) brought near to the mouth of tube in which the reaction was carried out turned slightly dark in colour.

Emulsions that were made using acasia, evolved hydrogen sulphide within five days, detected by the characteristic rotten egg smell and the turning of wet silver nitrate paper black. The extract suspended in 3% Tween 80, produced these effects after two months.

2.4 Helminths

The helminths used in this study were Nippostrongylus braziliensis (as a model for hookworms) and Hymenolepis nana (as a model for tapeworms).

2.4.1 Nippostrongylus braziliensis

The life cycle of N. braziliensis begins when eggs laid by the female worms pass into faeces of the host and reach external environment where they continue their development under favourable conditions of temperature and humidity. The first larvae emerge from the eggs after 24 hours. They moult twice in about five days giving second and third larvae stages. The last stage is the infectious form, capable of active penetration through the host's skin and arriving about 15 hours later in the lungs, reaching the lungs via the blood stream. Forty-five hours after infestation, the larvae reach bronchi then trachea before they are swallowed. They finally arrive in the intestine towards the 60th hour. There they become transformed into adults in about five days, after a fourth and last moulting. Laying of eggs begins immediately, and attain maximal level between the eighth and twelveth days following infestation, but eggs can be detected in faeces of parasitized rats from the sixth day (Cavier, 1973).

Infective larvae of N. braziliensis were obtained from the Department of Parasitology and

Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The larvae were recovered from the droppings of rats that had earlier been infected heavily with infective larvae using the method of Standen (1963).

The infective larvae obtained were put into a container, distilled water was added and the volume adjusted such that 0.2 ml will contain about 200 larvae. Using a large needle (18 gauge) and insulin syringe, 0.2 ml of the distilled water containing the larvae was injected into the cervical region of the rats.

After seven days, droppings recovered by squeezing them out of the rectum were examined qualitatively by floatation using 51% hydrated Magnesium sulphate as the floatation medium. Rats not shedding *N. braziliensis* ova and those shedding ova of other helminths were discarded from the experiment.

2.4.2 Chemotherapeutic Trials

Animals were grouped into four per group, and chemotherapeutic trials carried out on the tenth, eleventh and twelfth days after experimental ingestion with larvae. Magnesium sulphate at a dose of 4.6 g/kg body weight was found to effectively purge the animals and this dose was maintained throughout the experiments. Animals were fasted the night preceding the treatment and fed two hours after

treatment. The drugs were administered orally.

The extract was given at doses of 0.5 g/kg, 1.0 g/kg, 1.5 g/kg and 2.5 g/kg to the different groups once daily for three days. A similar dosage regimen of extract was given to other groups of animals once followed by magnesium sulphate two and twenty-four hours later. Each dose was adjusted with normal saline to 1 ml such that every animal received a volume of 1 ml. Two groups of animals served as controls, one group was given 1 ml of 3% Tween 80 while the other group received 1 ml of normal saline.

To compare the result with a standard drug, three groups of rats were given 100 mg/kg, 150 mg/kg and 200 mg/kg of bephenium hydroxynaphthoate respectively once daily for three days.

On the fourteenth day, the rats were fasted so as to empty the intestine as much as possible. They were killed on the fifteenth day and at autopsy, the first 15 cm of the small intestine was removed, cut open and pressed between two glass plates and examined for worms using dissecting microscope. The number of worms in each animal was recorded.

2.4.3 Hymenolepis nana

H. nana, the dwarf tapeworm, is found in the intestine of rat, mouse and man. Some workers believe that the mouse or rat parasite is a distinct subspecies, Hymenolepis nana fratena. There is little

doubt that the rat or mouse parasite can infect man. (de Carneri and Vita, 1973).

H. nana can be transmitted directly from one animal to another without the intervention of any intermediate host. Infective eggs shed by one animal get to the second animal through the mouth. The larvae quickly emerge once the eggs have been swallowed and penetrate into the villi where they grow for 3-4 days as cysticercoids. From the villi, they attach themselves to the walls of the small intestine where they grow into maturity. Fifteen days or so after infections, eggs first appear in the faeces, the majority of the adults are eliminated in the faeces within 30-40 days (de Carneri and Vita, 1973).

Mice (4-5 weeks old) weighing between 18-20g from the Animal House, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria were screened for ova of H. nana using 51% Magnesium sulphate as the floatation medium. Mice that showed H. nana ova in their stool were sacrificed, the small intestine cut off and the worms flushed out using a 30 ml syringe. The last three proglottids of the worms were cut off and tore apart to release eggs using a dissecting microscope. The ova were suspended in 9% saline and the volume adjusted such that 0.5 ml contained about 200 ova. Using a syringe, needle and catheter, young rats weighing between 70-100 g were dosed with about 200 ova by oral route.

On the fifteenth day, the rats were examined for ova. Those that showed positive ova in their stools were used for in vivo and in vitro experiments.

2.4.5 In Vivo Experiment

Rats were grouped into three animals per group, starved the night before the experiment and fed two hours after the treatment.

The extract was given at doses of 0.25 g/kg, 0.5 g/kg, 1.0 g/kg and 2.0 g/kg to different groups of the rats once daily, for three days. Similar dosage regimen was given to other groups of rats once, followed by a dose of magnesium sulphate two and twenty-four hours later. Rats in the first control group received 1 ml of 3% Tween 80 while those in the second control group were given 1 ml of normal saline. To compare the effect of the extract with a standard drug, three groups of rats were given niclosamide 100 mg/kg, 300 mg/kg and 600 mg/kg daily for three days.

Animals were fasted a day after the treatment and killed the second day. The whole of the small intestine was removed and the worms flushed out. The intestine was opened to remove any worms that stuck into the wall. The number of worms per animal was recorded.

2.4.6 In Vitro Experiment

The method recommended by de Carneri and Vita (1973) was followed. Rats harbouring H. nana were

fasted overnight and killed, their small intestines removed and the tapeworms flushed out with a 30 ml syringe using 9% saline.

The worms were washed twice and placed in nutrient broth consisting of peptone 10 g, yeast extract 2.5 g, sodium chloride 6 g, and distilled water to one litre. The pH was adjusted to 8.5 with ammonia solution. Immediately before use, crystalline penicillin (500 unit per ml) and streptomycin (0.1 mg. per ml) were added to the medium. After a maximum of three hours at 37°C, six worms were placed in each petri dish containing the nutrient broth and the desired concentration of the extract or niclosamide. Because the quantity of extract was small, it was diluted with arachis oil to raise the oily content to about 20% as recommended by Carter (1965). Petri dishes containing only nutrient broth and those containing 20% arachis oil emulsion served as control.

After twenty-four hours of incubation at 37°C, the worms in various petri dishes were stimulated with light, pin or both and the degrees of movement of the scolex and proglottids recorded.

2.5 Isolated Tissue Preparations

A Ugo Basile recording microdynamometer (model 7050) with appropriate transducer was used to record the mechanical activity of the various isolated muscle preparations. The isolated tissues were suspended in standard physiological fluids (appendix). The equilibrating period for the tissues was about 30 min.

2.5.1 Phrenic nerve-Diaphragm Preparation of the Rat (Bulbring, 1946)

Hemi-diaphragms together with their phrenic nerve, were removed from male rats weighing between 300 and 350g. Each preparation was set up in a 150 ml organ bath containing Krebs solution aerated with oxygen and maintained at a temperature of 37°C. A tension of 1 gm was applied to the tissue. The muscle was stimulated directly or indirectly through the phrenic nerve using electrodes. Maximal twitches were produced with square pulses of 5 ms duration and of supramaximal strength delivered from a Ugo Basile stimulator (model Cat. 3000) at a frequency of 0.4 Hz. The voltage for stimulating the nerve varied from 2.5 to 4 volts while the stimulating voltage for the muscle varied from 10 to 20 volts.

2.5.2 The Chick Biventer-cervicis Preparation (Ginsborg and Warriner, 1960)

The biventer cervicis muscles were removed from one to two week old chicks killed by ether inhalation. The tissues were suspended in 25 ml organ baths containing Krebs solution aerated with oxygen and maintained at a temperature of 37°C. A tension of 1 gm was applied to the tissues.

2.5.3 Rat Uterus Preparation

The rat uterus was prepared following the procedure described in the British Pharmacopoeia (1968).

Adult female rats 3-4 months old weighing between 140 and 150 g were pretreated with stiboestrol 0.1 mg/kg twenty-four hours before the experiment was carried out in order to bring the rats to oestrous. The animals were killed and the abdomen cut open to expose the uterine horns. The tissues were set up in 25 ml organ baths containing De Jalon's Solution, aerated with oxygen and the temperature maintained at 30°C.

2.5.4 Guinea-pig Isolated Atria (Alles and Ellis, 1948)

Paired atria were removed from male guinea-pigs weighing between 600 and 700 g and suspended in 25 ml organ bath containing Ringer-Locke Solution gassed with oxygen and maintained at 30°C.

2.6. Toxicological Study

Three days old chicks (35-38 g) and 5 weeks old mice (20-22 g) were dosed with the extract (2 g/kg) subcutaneously and observed for any lethal effects within 24 hours.

CHAPTER IIIRESULTS3.1 Extraction

The total weight of the dried seed used was 1.5 kilograms and 120.8 grams of the extract was obtained. The percentage yield was therefore 8.05. The extract was a pale yellow oily substance with spicy and pungent flavour.

3.2 Effect of Extract on Nippostrongylus braziliensis in vivo

The number of worms recovered from the animals was generally low compared to the average number of larvae infected. The average number of worm in the control animals was 52 ± 14 , that is only 19 to 33% of the larvae developed into worms. Table 1 shows the result of three sets of experiments and Fig. 3.1 shows the graphical representation of the results.

When the results were analysed using student's t-test, the effects of all dosage regimen of the extract were not significant.

With Bephenium hydroxynapthoate, a standard drug in the treatment of ancylostomiasis, however, the results were significant at a dose of 150 mg/kg ($P < 0.05$) and highly significant at 200 mg/kg ($P < 0.001$).

Table 1: Percentage deparasitization of rat infected with *N. braziliensis* and treated with *Carica papaya* seed extract or bephanium hydroxynaphthoate.

Drug and Dosage	Number of rats	Total worms at autopsy	Worms per rat Mean \pm s.e.m.	Percentage deparasitization
Control	12	618	52 \pm 14	
Extract at 0.5 g/kg once daily for three days.	11	480	44 \pm 9	15.38
Extract at 0.5 g/kg once followed by Magnesium sulphate 2 and 24 hours later.	10	497	50 \pm 13	3.85
Extract at 1.0 g/kg once daily for three days.	12	414	35 \pm 6	32.69
Extract at 1.0 g/kg once followed by Magnesium sulphate 2 and 24 hours later.	11	510	46 \pm 11	11.56
Extract at 1.5 g/kg once daily for three days	12	368	31 \pm 7	40.38
Extract at 1.5 g/kg once followed by Magnesium sulphate 2 and 24 hours later.	10	400	40 \pm 13	23.08

Table 1 (Cont'd.)

Drug and Dosage	Number of rats	Total worms at autopsy	Worms per rat Mean \pm s.e.m.	Percentage deparasitization
Extract at 2.0 g/kg once daily for three days	12	449	37 \pm 8	26.85
Extract at 2.0 g/kg once followed by Magnesium sulphate 2 and 24 hours later.	10	492	49 \pm 15	5.76
Bephenium hydroxynaphthoate 100 mg/kg once daily for three days.	12	320	27 \pm 3	48.08
Bephenium hydroxynaphthoate 150 mg/kg once daily for three days.	12	172	14* \pm 2	73.08
Bephenium hydroxynaphthoate 200 mg/kg once daily for three days.	12	75	6** \pm 2	88.46

* and ** indicate the group of rats in which the number of worms found were significantly ($P < 0.05$) and highly significantly ($P < 0.001$) different from the number found in the control group using student's t-test respectively.

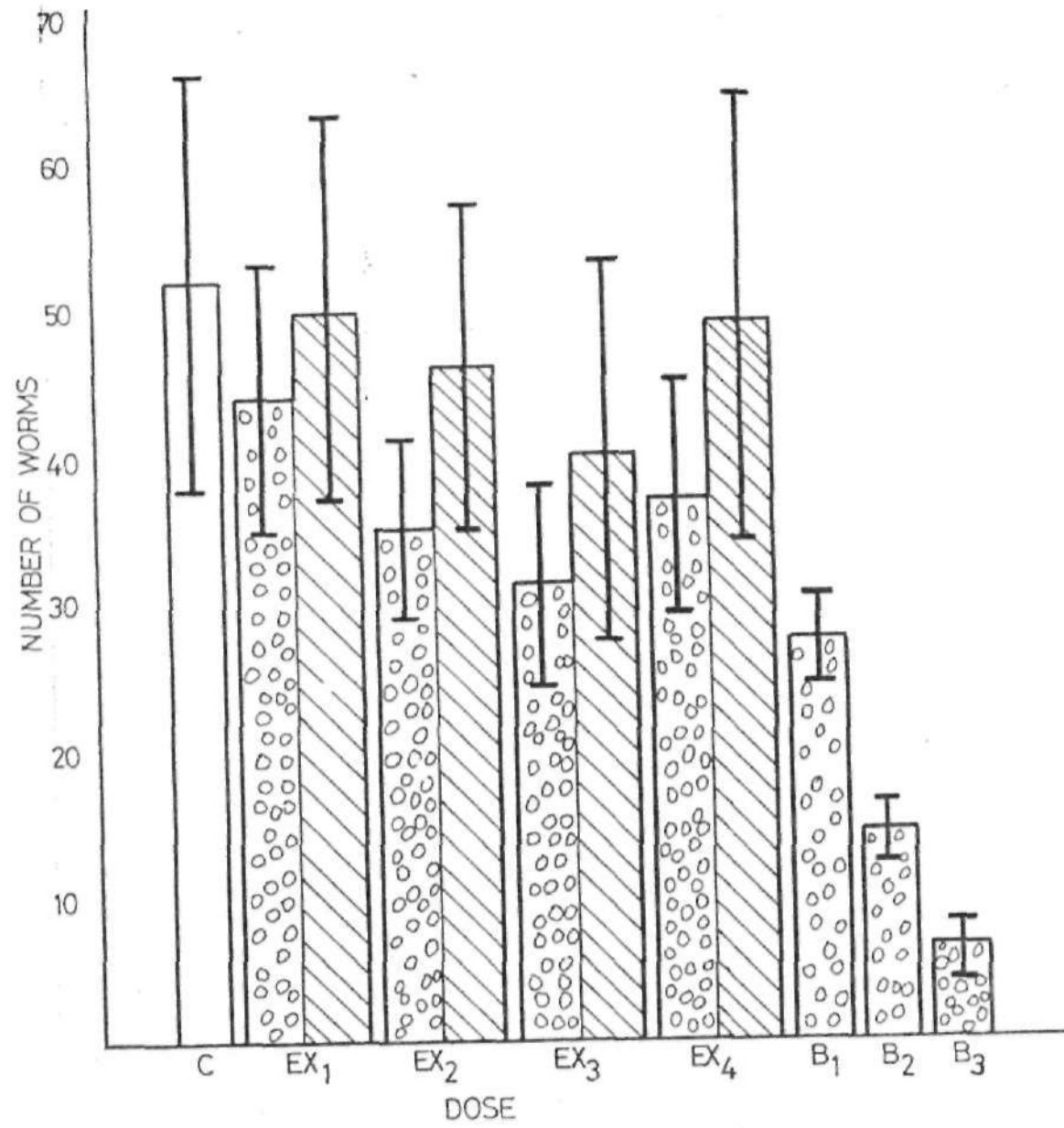





Fig. 3.1 Effects of *Carica papaya* seed extract (EX) and bephenium hydroxynapthoate (B) on *N. braziliensis* experimentally infected in rats. The histograms represent the mean \pm s.e.m. of the number of worms found in the rats.

EX₁ = 0.5 g/kg, EX₂ = 1.0 g/kg, EX₃ = 1.5 g/kg,
EX₄ = 2.0 g/kg.

B₁ = 100 mg/kg, B₂ = 150 mg/kg, B₃ = 200 mg/kg

 drug given once daily for three days.

 drug given once followed by magnesium sulphate 2 and 24 hours later.

 control (C).

3.3 Effects of Carica papaya Seed Extract on H. nana in vitro.

The extract at concentrations of 5.2, 3.9, 2.6, 1.3, 0.4, 0.2, and 0.1 mg/ml of the medium, none of the worms responded to stimulation. However, at 0.6mg/ml one out of the six worms responded. With niclosamide at concentrations of 15.5, 6.2 and 3.1 ug/ml, there was no movement of any of the worms when stimulated. At 1.9 ug/ml, one out of six worms responded to stimulation.

Table 2 shows the result obtained with lower concentrations of extract and niclosamide contained in medium.

All the worms in the nutrient broth that served as controls showed vigorous movement after stimulation. In the nutrient broth that contained various volumes (0.05 - 2 ml) of 20% arachis oil emulsion, all except one worm, showed vigorous movement on stimulation.

The concentration of the extract or niclosamide that either paralysed or killed 50% of the worms was found to be 35 ug/ml for extract and 120 ng/ml for niclosamide (Fig. 3.2.).

Table 2: Effects of *Carica papaya* Seed Extract or
Niclosamide on *H. nana* Incubated in
Nutrient Broth.

Drug	Concentrations(ug/ml) of drug in medium	Degrees of movement No. moved/ No. used.
Extract	50	1 ⁺⁺ /6
"	30	8 ⁺ /12
"	10	6 ⁺⁺⁺ /6
Niclosamide	1.9	1 ⁺ /6
"	0.9	1 ⁺⁺ /6
"	0.4	0/12
"	0.19	2 ⁺ /6
"	0.095	3 ⁺⁺ /6
"	0.048	6 ⁺⁺ /6

+, ++, and +++ indicate slight movements, movements,
and vigorous movements after stimulation respectively.

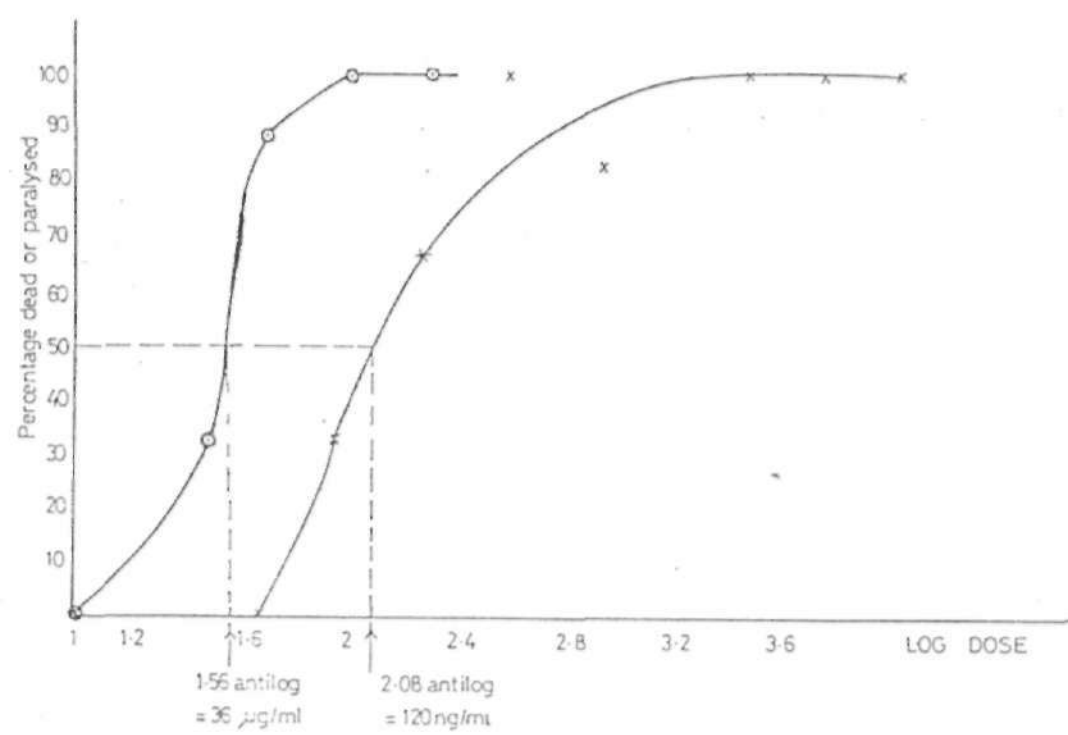


Fig. 3.2 Log-dose response of extract (—○—○—○) and niclosamide (—×—×—×) on *H. nana* in nutrient broth in vitro.

3.4 Effect of the Extract on *H. nana* in vivo

The experiment was repeated three times with three animals per group. The mean results of the three sets of experiments are shown in Table 3. Figure 3.3 shows the mean number of worms found at autopsy against the dose of extract or niclosamide.

The extract (0.5 g/kg) administered once daily for 3 days exhibited a significant activity against *H. nana* ($P > 0.05$). At doses of 1.0 g/kg and 2.0 g/kg once for three days, the effect of the extract was highly significant ($P > 0.001$). In single dose treatment with the extract (0.5 g/kg) followed by purging with magnesium sulphate, the activity was not significant but at 1.0 g/kg and 2.0 g/kg followed by purging the activity of the extract was highly significant ($P > 0.001$). Doses given for consecutive days were more effective than single doses followed by purging.

With niclosamide, all the three doses (150 mg/kg, 300 mg/kg and 600 mg/kg) used for three consecutive days were highly significant ($P > 0.001$). At 600 mg/kg of niclosamide, all the nine rats were completely deparasitized.

Table 3: Percentage deparasitization of rats infected with H. nana and treated with Carica papaya seed extract or niclosamide.

Drug and Dosage	Number of rats	Total worms at autopsy	Worms per rat Mean \pm s.e.m.	Percentage deparasitization
Control	14	304	22 \pm 3	
Extract at 0.25 g/kg once daily for three days.	9	173	19 \pm 4	13.64
Extract at 0.25 g/kg once followed by Magnesium sulphate 2 and 24 hours later.	8	164	21 \pm 3	4.55
Extract at 0.5 g/kg once daily for three days	9	116	13 \pm 1	40.91
Extract at 0.5 g/kg once followed by Magnesium sulphate 2 and 24 hours later.	9	134	15 \pm 2	31.82
Extract at 1.0 g/kg once daily for 3 days.	9	46	5 \pm 1	77.27
Extract at 1.0 g/kg once followed by Magnesium sulphate 2 and 24 hours later.	9	67	7 \pm 2	68.18

Table 3 (Cont'd.)

Drug and Dosage	Number of rats	Total worms at autopsy	Worms per rat		Percentage deparasitization
			Mean	\pm s.e.m.	
Extract at 2.0 g/kg once daily for three days.	9	19	2 \pm 1	1	90.90
Extract at 2.0 g/kg once followed by Magnesium sulphate 2 and 24 hours later.	8	22	3 \pm 1	1	86.36
Niclosamide 150 mg/kg once daily for three days.	8	47	6 \pm 2	2	72.72
Niclosamide 300 mg/kg once daily for three days.	9	20	2 \pm 1	1	90.90
Niclosamide 600 mg/kg once daily for three days.	9	0	0	0	100

* and ** indicate group of rats in which the number of worms found were significant ($P < 0.05$) and highly significant ($P < 0.001$) different from the number found in the control using student's t-test respectively.

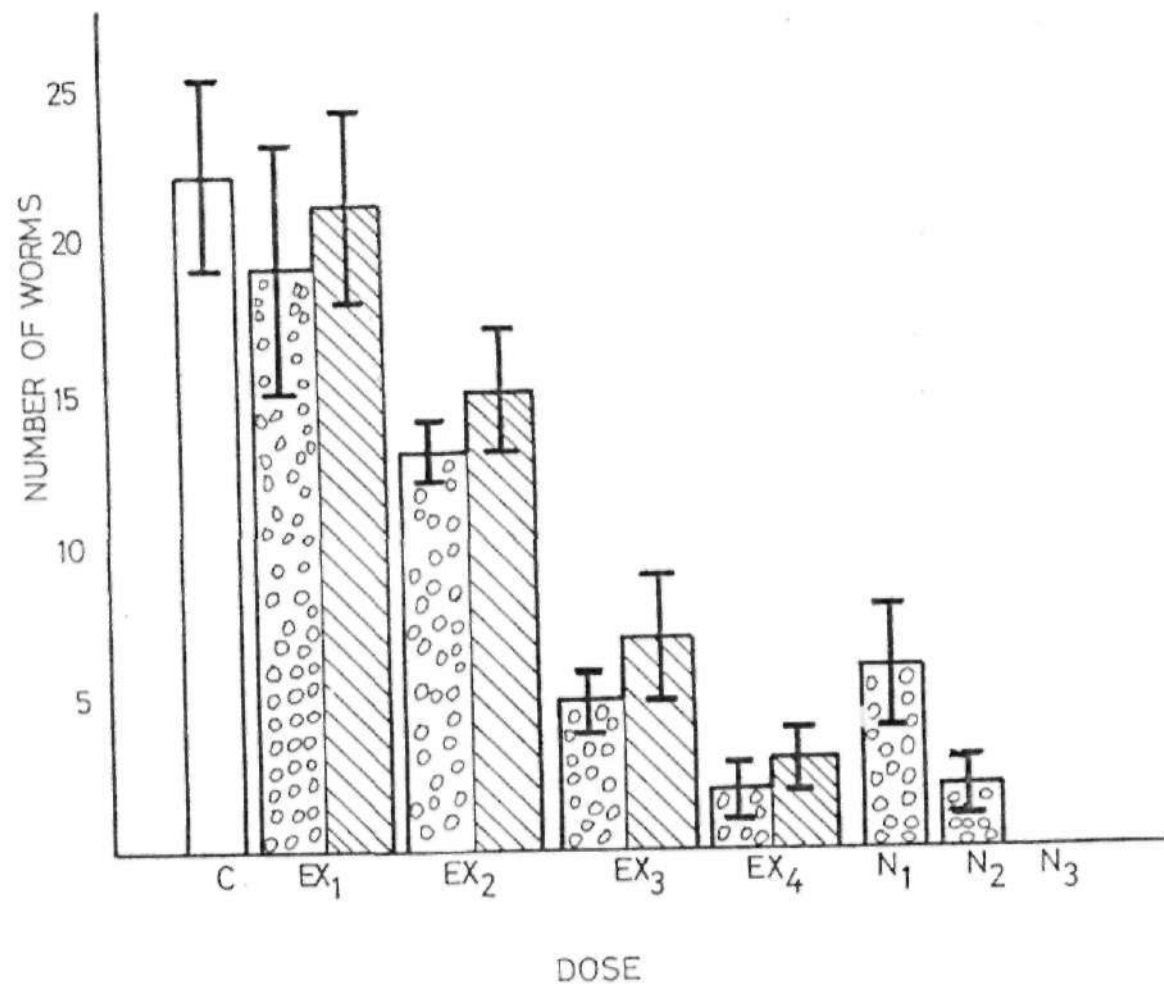





Fig. 3.3 Effect of *Carica papaya* seed extract (EX) or niclosamide (N) on *H. nana* experimentally infected in rats.

The histograms represent the mean \pm s.e.m. of number of worms found in the rats.

EX₁ = 0.25 g/kg, EX₂ = 0.5 g/kg, EX₃ = 1.0 g/kg,
 EX₄ = 2.0 g/kg, N₁ = 150 mg/kg; N₂ = 300 mg/kg,
 N₃ = 600 mg/kg.

-  drug given once daily for 3 days.
-  drug given once followed by magnesium sulphate 2 and 24 hours later.
-  control (C).

3.5 Isolated Tissues

3% Tween 80 and 2% acacia gum had no effect on all the isolated tissue preparations used.

3.5.1 Rat Phrenic Nerve-Diaphragm muscle Preparation

The effect produced by the extract was the same whether the muscle was stimulated directly or indirectly, through the nerve. At concentration of 0.02 mg/ml, the extract did not modify responses by the tissue after stimulation. However, higher bath concentrations (≥ 0.2 mg/ml) affected the tissue. The pattern of the response was an initial increase in the twitch amplitude, followed by an upward shift in base line and a diminishing amplitude. Finally, the tissue went into permanent contracture. Once the contracture process had started, washing the tissue did not reverse it (Fig. 3.4 and 3.5).

Both D-Tubocurarine (2 ug/ml) and verapamil (0.2 mg/ml) had no effect on the contracture.

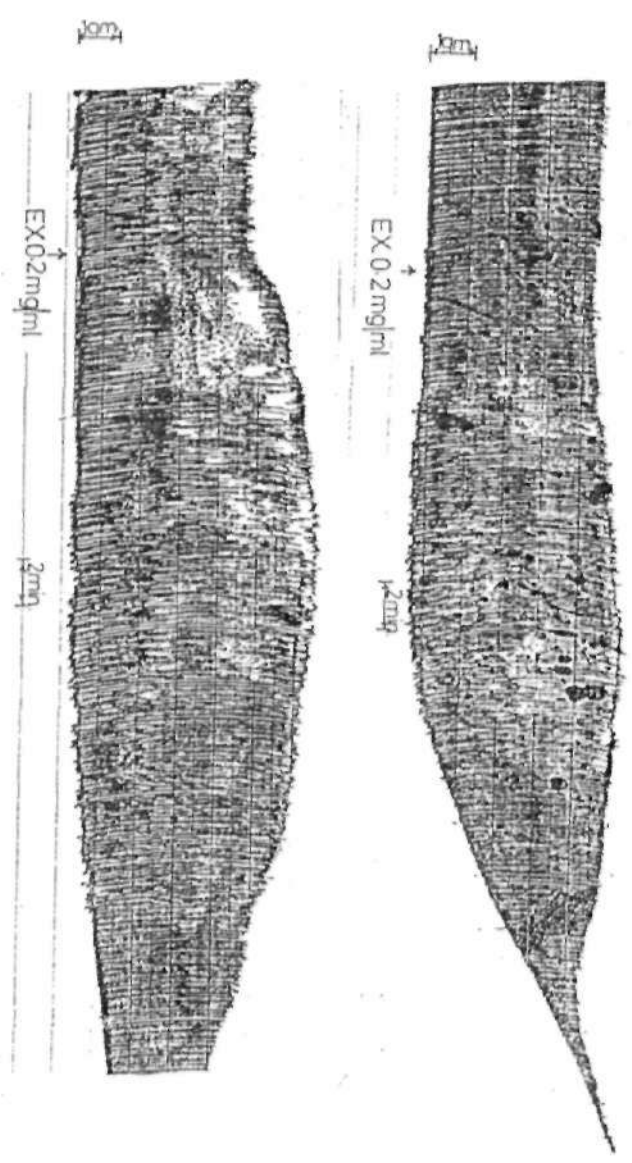


Fig. 3.4 Effect of Carica papaya seed extract on rat phrenic nerve-diaphragm preparation.

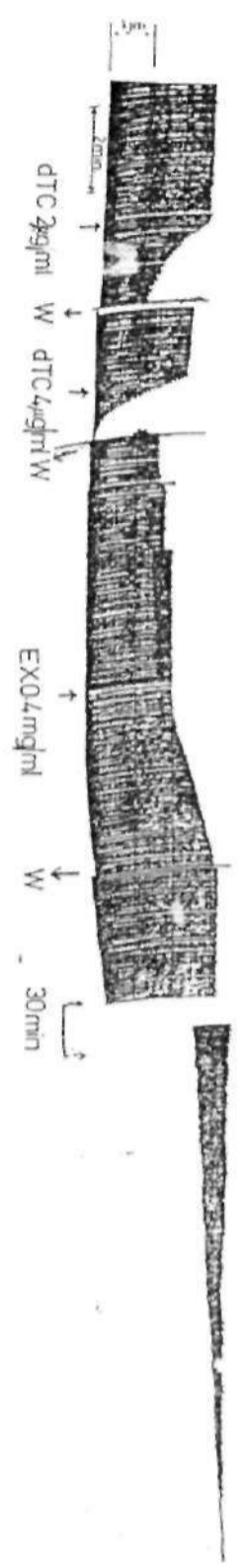


Fig. 3.5 Effect of Carica papaya seed extract on rat phrenic nerve-diaphragm preparation.
dtc = D-Tubocurarine, EX = extract, W = washing.

3.5.2 Chick Biventer-Cervicis Preparation

The extract produced a sustained contracture and the effect was not reversible after washing (Fig. 3.6). The higher the concentration the shorter the onset of the contracture. At bath concentrations above 960 ug/ml some of the preparations snapped at the point of attachment to the organ bath. 32 ug/ml of the extract left in the bath for two hours produced the same effect as higher concentrations (Fig. 3.7 panel A).

8 ug/ml of the extract did not produce any noticeable effect even after being in contact with the tissue for two hours. (Fig. 3.7 panel B). D-Tubocurarine (3.2 ug/ml - 12.8 ug/ml) did not inhibit the contractile effect of 32 ug/ml of the extract.

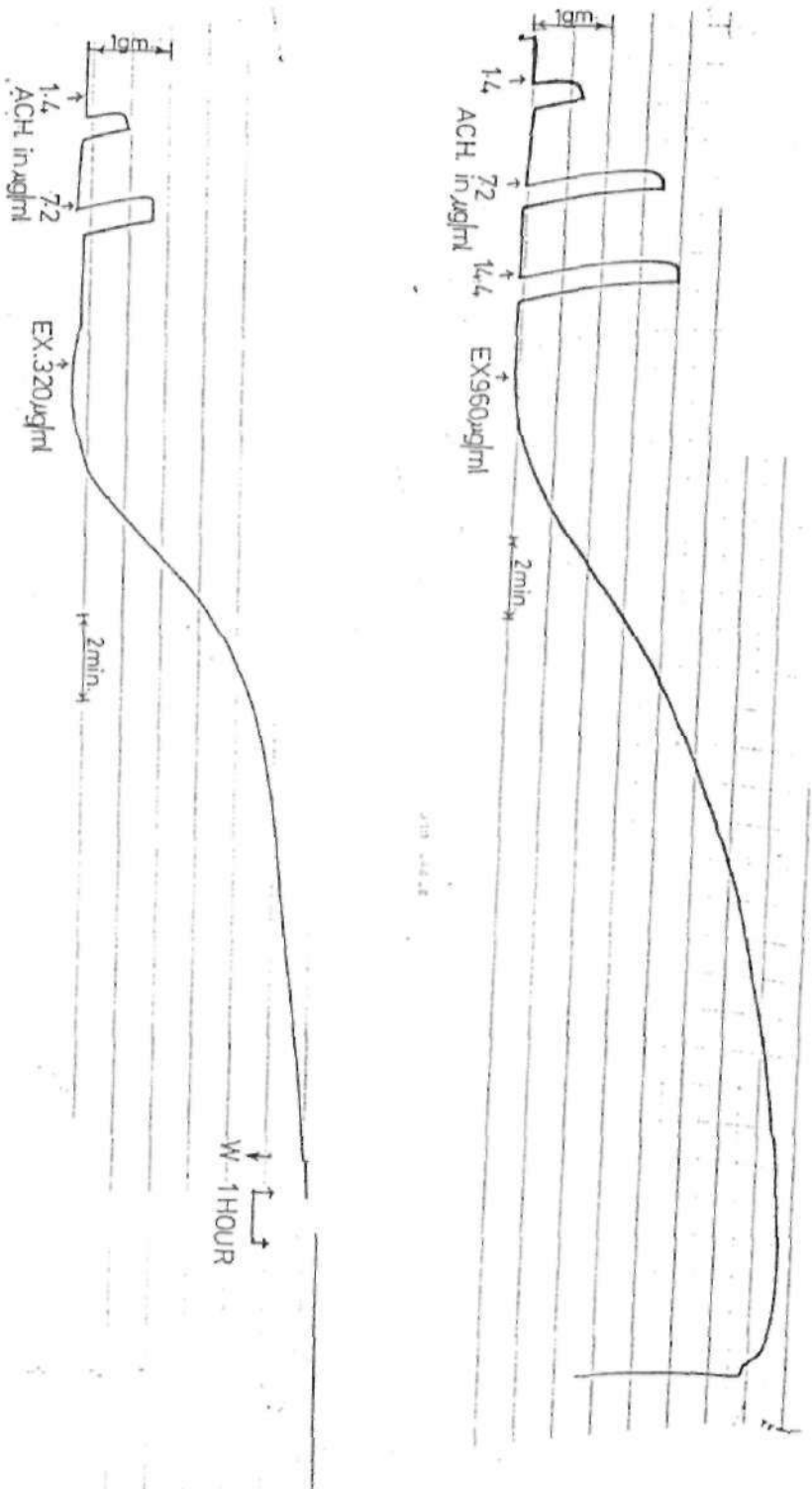


FIG. 3.6 Effect of *Carica papaya* seed extract on chick biventer cervicis muscle preparation.
 ACH = acetylcholine, EX = extract, W = washing.

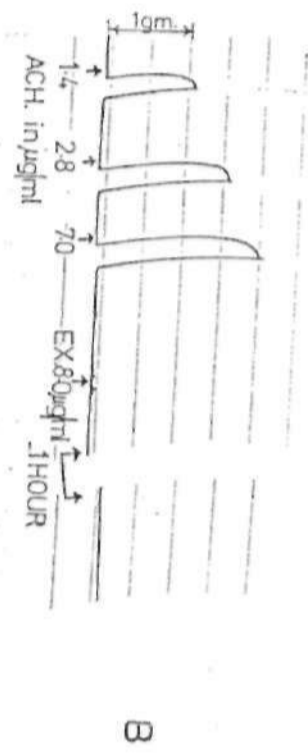


Fig. 3.7 Effect of *Carica papaya* seed extract on chick biventer cervicis muscle preparation.

Panel A Response of tissue to the extract (32 ug/ml) in presence D-Tubocurarine (9.4 ug/ml).

Panel B Effect of the extract (8 ug/ml) on the preparation.

3.5.3 Rat Uterus Preparation

0.5 ng/ml of the extract induced rhythmic contractions of both oestrous and non-oestrous rat uterus. The contractions were accompanied by an upward shift of the base line (Fig. 3.8).

The base line returned to normal whether the preparations were washed or not. It was observed that oxytocin (30 ng/ml) which produced contractions of the uterus failed to contract uteri exposed to the extract. The same tissue also failed to respond to a second dose of the extract (Fig. 3.8). Concentrations of the extract (≤ 20 ug/ml) did not produce any effect on the tissue.

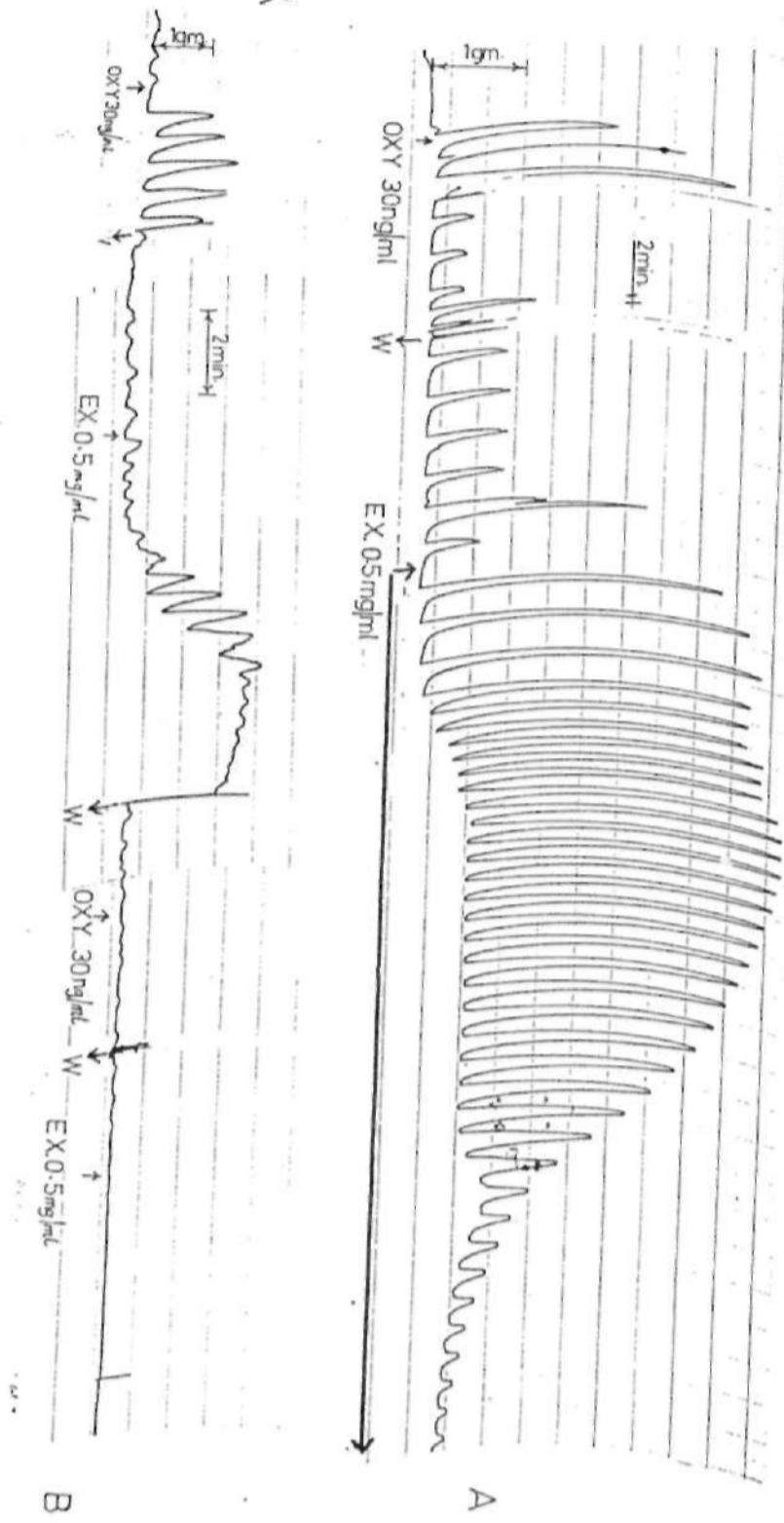


Fig. 3.8 Effect of *Carica papaya* seed extract on rat uterus.
 Oxy = oxytocin, EX = extract, W = washing.

3.5.4 Guinea-Pig Isolated Atria

The extract produced an initial positive chronotropic and inotropic effects on the spontaneously beating guinea-pig isolated atria. The extract (0.2 mg/ml) increased the amplitude from 14 ± 0.9 mm to 19 ± 1.4 mm. The increase was statistically significant ($P < 0.05$). The same dose of the extract increased the heart beats per min from 134 ± 3 to 137 ± 5 . This was not statistically significant. The initial effects were followed by a progressive decrease in both amplitude and heart beats per min. The decrease was not affected by washing the tissues. (Fig 3.9 Panel (B)).

Tissues exposed to the extract failed to respond to acetylcholine (15 ng/ml - 1.7 ug/ml), adrenaline (0.3 ug/ml - 3.6 ug/ml), or a second dose of the extract (Fig. 3.9 panel A)

Table 4 shows changes in heart beats per min and amplitude one hour after tissues were exposed to the extract. The decrease in both heart rate and amplitude were highly significant ($P < 0.001$). This decrease continued progressively until the atria were arrested.

Table 4. Effects of Carica papaya seed extract (0.2 mg/ml) on the rate and amplitude of isolated guinea-pig atria.

atria	Before addition of extract		After addition of extract		One hour after washing off extract	
	rate (beats per min)	amplitude (mm)	rate (beats per min)	amp. (mm)	rate (beats per min)	amp. (mm)
1	129	13	130	20	86	4
2	128	11	128	16	118	6
3	140	15	153	24	93	5
4	141	15	143	17	82	9
5	130	16	132	20	65	8
Mean \pm s.e.m	134 \pm 3	14 \pm 0.9	137 \pm 5	19 \pm 1.4	89 \pm 9	7 \pm 1.0

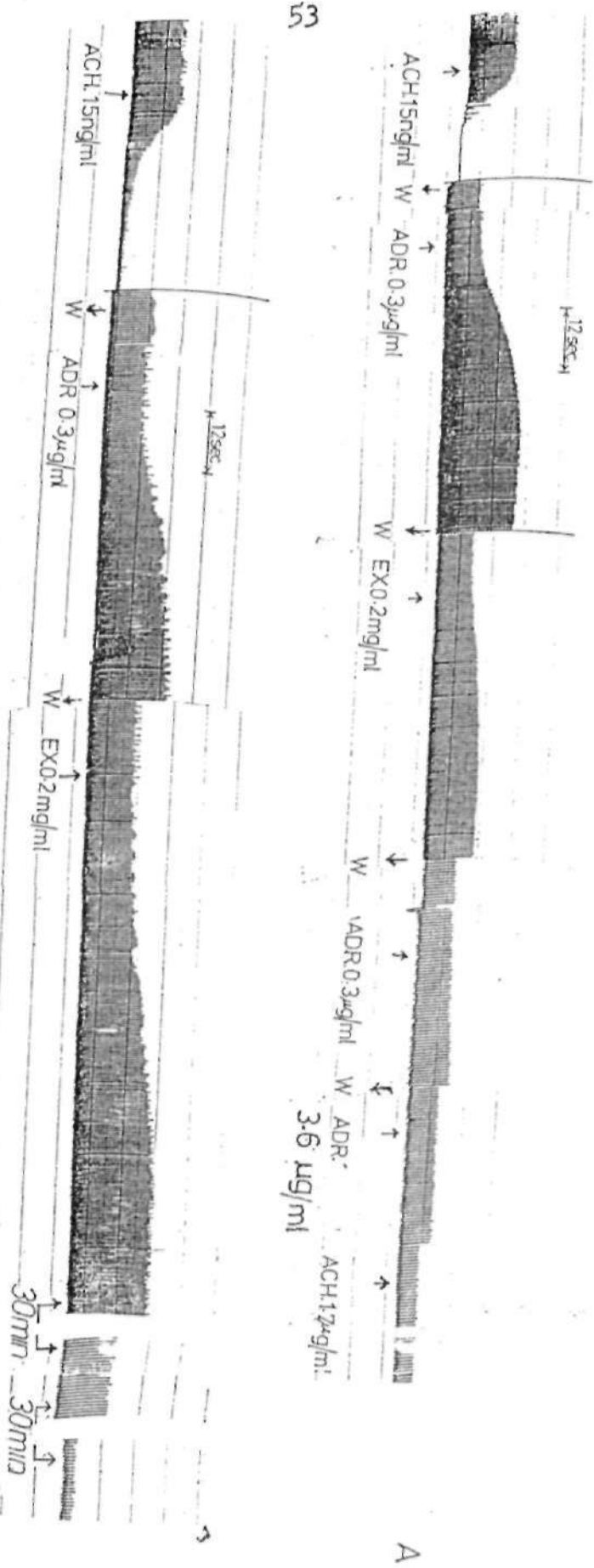


Fig. 3.9 Effect of *Carica papaya* seed extract on isolated guinea-pig atria.

Panel A Application of adrenaline (3.6 µg/ml) and acetylcholine (1.7 µg/ml) after exposure of atria to the extract.

Panel B Extract in contact with tissue for one hour.

3.6 Toxicological Study :

A dose of 2 g/kg body weight injected into five mice and five chicks did not produce any noticeable adverse effects.

CHAPTER IVDISCUSSION

Chan, Heu, Tang, Okazaki and Ishizaki (1978), working with Solo variety of Carica papaya obtained nine different fatty acids after extraction with diethyl ether. Chan et al., (1978) reported that the BITC content of the oil was 0.56% w/w. It is possible that the composition of the Nigerian Carica papaya seed extract may differ from the Solo variety reported by Chan and his co-workers (1978). They described the colour of their extract as light green while the extract from the Nigerian Carica papaya was pale yellow. However, the spicy and pungent flavour agrees with earlier work reported by Heid and Curl (1944). Ellinger and Hodgkin (1956) attributed the spicy pungent flavour to the presence of BITC which has been shown by Tang (1973) to be formed from benzyl glucosinolate by the action of thioglucosidase.

The extract undergoes hydrolysis which according to Vogel (1974) is common to mustard oils, to which BITC belongs, releasing H_2O and carbon dioxide. In the present study, emulsions of extract using Acasia gum as emulsifying agent underwent a reaction releasing hydrogen sulphide after 5 days, and in 3% Tween 80 after 2 months. Tang, Bhothiapaksa and Frank (1972) reported a bacterial degradation of BITC yielding hydrogen sulphide. From the foregoing, it is possible that the extract obtained in this study

contains BITC among other possible constituents.

The maximum dose of the extract used against N. braziliensis was 2.0 g/kg once daily for three days. The percentage deparasitization was 28 and the result was not statistically significant ($P > 0.1$). According to Cavier (1973), a 50% deparasitization can be regarded to be significant. Similarly, at a dose of 2.0 g/kg followed by purging, the animals were not significantly deparasitized. The highest deparasitization obtained in treatment against N. braziliensis was at a dose of 1.5 g/kg once daily for three days (40.38%) which was not significant statistically. Therefore, a reduction in number of worms in this group of rats may not be due to the effect of the extract on N. braziliensis.

Bephenium hydroxynapthoate at doses of 100 mg/kg, 150 mg/kg and 200 mg/kg, once daily for three days produced 48.08%, 73.08% and 88.46% deparasitization respectively. These values are close to those obtained by Cavier and Lahmani (1963) with corresponding doses. At doses of 150 mg/kg and 200mg/kg, the results were significant ($P < 0.05$) and highly significant ($P < 0.001$) respectively.

The extract exhibited activity on H. nana both in vitro and in vivo. At concentrations greater than 100 ug/ml, the worms did not respond to stimulation while concentrations below 10 ug/ml had no effect on the worms. The LD_{50} of the extract against the worms was 36 ug/ml and that of niclosamide was 120 ng/ml.

Worms not responding to stimulation could be considered either dead or paralyzed. The worms did not respond to stimulation in niclosamide concentrations of above 190 µg/ml. This value is close to that of de Carneri and Vita (1973) that niclosamide (0.1 - 10 mg/ml) which is characterized by its rapid action, paralyzes H. nana within one hour.

In vivo results showed the activity of the extract to be significant at 0.5 g/kg and highly significant at 1.0 g/kg once daily for three days. Similarly, the single dose treatment followed by purgation was significant at 1.0 g/kg and highly significant at 2.0 g/kg. The results of the two types of treatment do not reveal whether the extract is a taenicide or a taenifuge. This is because with a taenifuge the worms are paralysed and expelled either by peristalsis or purgation. With a taenicide, the worms are either killed and expelled or digested. However, since higher doses of treatment gave a higher deparasitization, it favours the possibility that the extract may be a taenicide. Katiyar and Sen (1971) working with some synthetic isothiocyanates on H. nana reported that single dose of diphenylsulphide-4-4' diisothiocyanate (100 mg/kg) completely deparasitized rats infected with H. nana.

This results shows that Carica papaya seed extracts and indeed isothiocyanates have potential therapeutic use as cestocidal agents but may not be active in ancylostomiasis.

Drugs used in the chemotherapy of helminthiasis, according to Rollo (1980) either interfere with the metabolic pathways of the helminth (e.g. mebendazole, niclosamide, niridazole, pyrvinium, antimony compounds) or neurohumoral transmission (e.g. metrifonate, piperazine, pyrantel) or both (e.g. tetramisole and levamisole). The results obtained with phrenic nerve diaphragm and chick biventer cervicis muscle preparations indicate that the extract may not be acting at neuromuscular junction. It may be interfering with the process involved in contraction and relaxation beyond the neuromuscular junction.

The theory that explains contraction and relaxation of muscle fibres is what has been accepted by most physiologists as the sliding filament theory of contraction by Huxley (1964) and Huxley (1965). According to this theory, changes in length of muscle fibres are caused not by changes in length of filaments, but by filaments sliding over another.

Propagating action potential can be evoked in a muscle cell membrane either by applying stimulating electrodes directly to the muscle or by stimulating its motor nerve which in turn releases acetylcholine. The released acetylcholine diffuses across the narrow junctional gap and depolarizes post-junctional membrane to produce end plate potential. The action potential is transmitted to all myofibrils via the

T-tubes. According to Bowman and Rand (1980), electrical changes in membrane of T-tubules causes the release of small amount of so-called "triggered Ca^{2+} " from triadic junctions. In turn, this causes the release of "activator Ca^{2+} " from the lateral sacs and vesicles of sarcoplasmic reticulum, in which it is stored into the sarcoplasm in the region of the myofilaments. The released Ca^{2+} from the sarcoplasmic reticulum diffuses to myosin and actin filaments and binds to troponin-tropomyosin-actin complex leading to activation of myosin ATPase and ATP is hydrolysed consequently leading to formation of cross-linkages between actin and myosin and the sliding of myosin and actin producing shortening.

At rest, the amount of free Ca^{2+} in the sarcoplasm is low and the troponin-tropomyosin complex, together with Mg^{2+} and intact ATP prevent the formation of cross-linkages between myosin and actin so that relaxation is maintained.

After contraction, for restoration of relaxation, Ca^{2+} has to be removed from the troponin-tropomyosin complex which then can re-exert its inhibitory action. This is accomplished because the sarcoplasmic reticulum actively sequesters the Ca^{2+} . Muscle mitochondria also have a large capacity to store Ca^{2+} , although their affinity for it is lower than that of the sarcoplasmic reticulum and they accumulate calcium more slowly. The relative

importance of mitochondria to be involved in controlling the intracellular concentration of free Ca^{2+} depends on different types of muscle and the number of mitochondria present.

In addition to its role in active uptake of Ca^{2+} by sarcoplasmic reticulum, ATP plays other roles in relaxation process. It is needed to alter the binding capacity of the lateral sacs of sarcoplasmic reticulum in favour of Ca^{2+} over other ions. It has been suggested that ATP possibly produces this alteration by inducing a conformational change in the binding sites. Even when all the activation Ca^{2+} has been removed from myofilament, relaxation does not occur unless Mg^{2+} and intact ATP are present. These substances exert a direct action on the myofilament which is necessary in addition to ions of Ca^{2+} from troponin-tropomyosin complex to allow relaxation to occur. Relaxation therefore also involves the synthesis of ATP (Bowman and Rand, 1980).

From the foregoing, skeletal muscle can go into permanent contracture by altering any of the processes involved in the removal of Ca^{2+} from the myofilament, sequestering it into sarcoplasmic reticulum and mitochondria and binding it electrostatically to the storage sites. All these involve interference with ATP. Calcium ions are actively sequestered and this requires the hydrolysis of ATP

by sarcoplasmic reticulum ATPase. Inhibition of this enzyme or uncoupling of its activity will result in failure to sequester Ca^{2+} . Secondly, failure to free Ca^{2+} from troponin-tropomyosin complex makes Ca^{2+} permanently available. This could be through interference with Mg^{2+} or intact ATP, which could be brought about in the failure to resynthesize ATP.

At the storage site, ATP is involved in altering the binding capacity of the lateral sacs in favour of Ca^{2+} , if the binding capacity is altered, either by interference with ATP or the extract occupying the binding sites, then Ca^{2+} will not be effectively bound. Bowman and Rand (1980) have indicated that the sites at which Ca^{2+} is bound contains imidazole groups, phosphatidyl, serine and lecithin. The extract may interfere with any of these chemical groups at the binding sites.

It was observed that whether the skeletal muscle was electrically stimulated (as in phrenic-nerve diaphragm preparation) or not stimulated (as in chick biventer cervicis), a permanent contracture was produced. It is therefore likely that the extract is acting at the storage site, releasing Ca^{2+} . When the tissue was stimulated electrically, there was an initial increase in twitch amplitude which suggests that the intensity of contraction was increased, which may be due to a sudden increase in the availability of Ca^{2+} for the contractile process. This Ca^{2+}

may be coming from the sarcoplasmic reticulum. Since a similar effect was obtained in calcium free Kreb's Solution it can be suggested that the effect was not due to external calcium.

If the extract acts directly at the storage site to release Ca^{2+} then its mechanism is different from that of ryanodine, an alkaloid from the plant Ryania speciosa which produces failure of relaxation in isolated stimulated skeletal muscle. The effects of ryanodine are due to the alkaloid impairing the ability of sarcoplasmic reticulum to sequester Ca^{2+} (Bowman and Rand, 1980).

Other drugs that have been known to affect Ca^{2+} in the sarcoplasmic reticulum and mitochondria at high doses are caffeine, quinine and quinidine. Caffeine releases free Ca^{2+} from the sarcoplasmic reticulum. It also reduces the rate and capacity of Ca^{+} accumulation by isolated sarcoplasmic reticulum and mitochondria. Quinine releases both bound and free Ca^{2+} from the sarcoplasmic reticulum, while quinidine blocks the uptake of Ca^{2+} into the mitochondria (Bowman and Rand, 1980).

It has also been reported that certain anions such as thiocyanate ($\text{S}^{-} - \text{C}\equiv\text{N}$), iodide, nitrate and bromide act probably on the T-tubules whereby the transfer of excitation to sarcoplasmic reticulum is facilitated resulting in the release of Ca^{2+} from the

sarcoplasmic reticulum at smaller than normal level of depolarization.

The observed effect of the extract on both oestrous and non-oestrous uteri was that of contraction. Since the contractile effect was observed in both oestrous and non-oestrous rat uteri, it is likely that the action of the extract is not dependent on progesterone-oestrogen balance, unlike oxytocin which is known to stimulate both frequency and force of contractile activity which are highly dependent on the presence of oestrogen (Csapo, 1959).

Since second doses of both the extract (0.5 mg/ml) and oxytocin (30 mg/ml) failed to produce responses after the tissue had been exposed to the extract, it is likely that the extract interferes with the contractile process in the smooth muscle.

The structural basis of contraction of the filament sliding theory in vertebrate smooth muscle is not clear (Axelsson, 1970), though it is known that these muscles contain both actin and myosin and the actin is present in filament form. Some workers maintain that there is sufficient evidence supporting the possibility that the sliding filament theory may also be applicable to vertebrate smooth muscles (Neeham and Shoenberg, 1967; Shoenberg, 1969). However, the involvement of intracellular Ca^{2+} appears to be a problem. This is because the

development of endoplasmic reticulum, which corresponds to sarcoplasmic reticulum of the skeletal muscle, in different smooth muscle types varies greatly. In many smooth muscles it appears to be very poorly developed or even absent (Burnstock, 1970). Bauer, Goodford and Hüter (1965) indicated that many smooth muscle cells have a very small diameter (3-4 μ). Consequently, extracellular calcium is not far from the contractile mechanism. Ebashi (1961) proposed that contraction and relaxation of smooth muscle might be related to a shift of Ca^{2+} towards and away from the contractile elements.

It is therefore likely that the extract interferes with or prevents these fluxes of Ca^{2+} hence when this is established, application of agonist (e.g. oxytocin) or extract the second time will have no effect on the tissues.

The effect of the extract on the guinea-pig atria was an increase in the force of contraction, accompanied by an increased heart rate. These initial effects were followed by a progressive decrease in both the force and rate of contraction. While the force of contraction and rate were decreasing, application of acetylcholine (15 ng/ml) that had abolished both the beats and amplitude before the use of the extract failed to produce any noticeable effect even when a dose as high as a hundred fold (1.7 μ g/ml) was used later. Similarly

the positive inotropic and chronotropic effects of adrenaline were not observed after the tissues had been exposed to the extract.

Effects of drugs on the heart can generally be explained by their influence on ionic distribution and movement across the cardiac membrane. As in all other excitable tissues, there is more of Na^+ and Cl^- extracellularly than intracellularly and more of K^+ intracellularly. There is also more of Ca^{2+} extracellularly. Ion fluxes associated with the heart have been divided into five phases, mainly phases 0, 1, 2, 3 and 4. Phase 0 involves the inrush Na^+ to the cell as a result of Na^+ gates being opened when the membrane is depolarized from the resting membrane potential (-90 mV) to -70 mV. At the peak, the membrane potential is reversed to a value of about +30 mV (Bowman and Rand, 1980). It is also accepted that in addition to rapid inward flux of Na^+ current during phase 0, there is inward flux of Ca^{2+} to a small extent (Beeler and Reuter, 1970). At the peak of the depolarization (+30 mV), Na^+ gates are closed and repolarization begins immediately which constitutes phase 1. The repolarisation is interrupted by phase 2 a secondary slower inward current of Ca^{2+} which gives rise to plateau of action potential. This is followed by phase 3 which is a further repolarisation resulting in K^+ efflux to resting potential. The

interval between resting potential is designated stage 4, in which there is efflux of Na^+ and influx K^+ as a result of Na^+-K^+ pump.

Acetylcholine abolishes contraction as a result of effect on sinoatrial node in increasing permeability to K^+ thereby hyperpolarising the membrane. Secondly it has a direct negative inotropic effect on the atrial myocardium which appears to be due to reduced uptake of Ca^{2+} during the plateau of action potential in the presence of acetylcholine (Bowman and Rand, 1980). It is claimed that the muscarinic receptors are coupled to guanylate cyclase in such a way that the stimulation of the receptors by acetylcholine increases the activity of the enzyme, and thereby increases the rate of formation of cyclic guanosine monophosphate (cyclic GMP) from guanosine triphosphate (GTP). The cyclic GMP affects the activity of transport and binding site for Ca^{2+} reducing the amount of Ca^+ available for contractile mechanism hence lowering the force of contraction (Bowman and Rand, 1980).

The positive inotropic effect of adrenaline has also been attributed to adrenaline activating adenylate cyclase which leads to an increase in the rate of formation of cyclic adenosine-3-5-monophosphate (Cyclic AMP), which activates a kinase enzyme that in turn activates some mechanism that makes more Ca^{2+} available.

Since after exposing the atria to the extract, both acetylcholine and adrenaline failed to produce any effect, it is likely that the two enzyme systems, cyclic GMP and cyclic AMP might have been affected such that they might be incapable of mobilizing Ca^{2+} . The initial increase in amplitude and heart beats could be related to the effect of the extract on Ca^{2+} . The extract could facilitate the release of calcium ions from sarcoplasmic or mitochondrial stores, an action similar to what the cardiac glycosides do during excitation (Kaus and Lee, 1969; Lee, Hong and Kang, 1970) so that more Ca^{2+} is available for contractile system. Alternatively, the extract could prevent Ca^{2+} from being sequestered into sarcoplasmic reticulum and thereby making it more available for the contractile process. However, since the tissues did not go into permanent contracture (no upward shift in base line) as the skeletal muscle preparations, there could be no continuous availability of calcium for sustained contracture. It therefore favours the argument that the extract could be acting via other mechanisms, cyclic GMP and cyclic AMP may be the possible candidates for the cardiac action of the extract.

The detection of hydrogen sulphide from the extract suggests that BITC may be present in the extract. However, the observed pharmacological

actions of the extract on isolated tissues are not consistent with an earlier report on BITC. El-Tayeb *et al.*, (1974) used petroleum spirit extract and observed that BITC had no effect on the rat phrenic-nerve diaphragm preparation.

The extract at a dose of 2 g/kg did not produce any noticeable effect on mice and chicks. This agrees with the report of El-Tayeb *et al.*, (1974) that there is no fatality with BITC (0.2 ~ 2.0 g/kg). It is likely therefore that the extract is relatively non-toxic on acute basis.

Further work needs to be done to find out if BITC is responsible for the observed pharmacological effects or any other components of the extract, since the pharmacological effects of BITC is lacking.

Furthermore, since the extract affects skeletal, smooth and cardiac muscles, a complete elucidation of its mechanism of action may extend the present knowledge of the process involved in contraction-relaxation coupling.

SUMMARY AND CONCLUSION

Diethyl ether extract of Carica papaya seeds has a significant activity against H. nana. Qualitatively, BITC was detected and the activity of the extract against H. nana could be attributed to the presence of BITC. The extract had no activity against N. braziliensis. The earlier reported anthelmintic property of Carica papaya seed (in Ascaridae) could be extended to cestodes (mainly H. nana) but not to hookworms.

The extract affects isolated skeletal muscle preparations directly resulting in permanent contracture. It contracted and desensitized the uterine smooth muscle to other agonists. On the cardiac muscle, it produces initial positive chronotropic and inotropic effects followed by a gradual decrease in force and rate of contraction of the heart. It also desensitized the heart to acetylcholine and adrenaline.

REFERENCES

- (1) AKEREJOLA, T.W., Van Veen, S., and Njoku, C.O. (1979).
Ovine and caprine diseases in Nigeria.
Bulletin of Animal Health and Production
in Africa, 27: 65-70.
- (2) ALLES, G.A. and Ellis, C.H. (1948).
Comparative actions of certain compounds
like alpha-fagarine
Journal of Pharmacology, 94: 416-425
- (3) ARNOLD, H.L. (1972).
Immediate treatment of insect stings.
Journal of the American Medical Association,
22: 341.
- (4) ARTHUR, R.P. and Shelley, W.B. (1955).
The role of Proteolytic enzymes in the
production of pruritus in man. Journal of
Investigatory Dermatology 25: 341.
- (5) AXELSSON, J. (1970).
Mechanical Properties of Smooth Muscle,
and the Relationship between Mechanical
Electrical Activity, In: Bulbring, E.,
Brading, A.F., Jones, A.W. and Tomita, T.
Smooth muscle pp. 289-315.
Edward Arnold Publishers, London.
- (6) BALLS, A.K. (1941).
Protein digesting enzymes of Papaya and
Pineapple. United States Department of
Agriculture, December, Circular No. 631,
Washington, D.C.
- (7) BARGE, G. (1910).
The Alkaloid of Carica papaya: Carpaine
Journal of Chemical Society 97: 466-469.
- (8) BARGE, G. Robinson, R. and Work, T.S. (1937).
Constitution of Carpaine. Journal of
Chemical Society, 1937: 711-713.
(Chemical Abstract, 31: Abstr. 53547,
(1937).
- (9) BAUER, H., Goodford, P.J. and Hüter, J. (1965).
The calcium content and ⁴⁵Calcium uptake
of smooth muscle of the guinea-pig taenia
coli. Journal of Physiology, 176: 163-179.

- (10) BEECHER, W. (1931).
Hyperesthetic rhinitis and asthma due to digestive ferment. Illinois Medical Journal, 59: 343.
- (11) BEELER, G.W. Jr. and Reuter, H. (1970).
The Relationship between membrane potentials, membrane currents and activation of contraction in ventricular myocardia fibres. Journal of Physiology, 207: 211.
- (12) BOSE, B.C. Saifi, A.Q., Vijayavargiya, R. and Bhagwat, A.W. (1961). Pharmacological Study of Carica papaya seeds with special reference to its anthelmintic action: Preliminary report. Indian Journal of Medical Science, 15: 888-892.
- (13) BOUM, B. Poussët, J.L., Lemonnier, F. and Hadchouel, M. (1978). Action des extraits de Carica papaya sur un ictere experimental cree chez le rat des saponosides Provenant du Brenania brierii. Toxicology and Applied Pharmacology 46: 353-362.
- (14) BOWMAN, W.C. and Rand, M.J. (1980).
Textbook of Pharmacology. 2nd Edition. PP. 171-175, 221-227. Blackwell Scientific Publications, Oxford.
- (15) BULBRING, E. (1946).
Observation on the isolated Phrenic nerve-diaphragm Preparation of the rat. British Journal of Pharmacology and Chemotherapy 1: 38-61.
- (16) BURDICK, M.E. (1971).
Carpaine; An alkaloid of Carica papaya its chemistry and pharmacology. Economic Botany, 25: 363-365.
- (17) BURNSTOCK, K.G. (1970).
Structure of Smooth Muscle and its Innervation. In: Bulbring, E., Brading, A.F., Jones, A.W. and Tomita, T. Smooth Muscle. Edward Arnold Publishers, London. PP 1-69.
- BRITISH Pharmacopoeia (1968).
Rat uterus method. Appendix xvii, The Pharmaceutical Press, London, PP. 1342.
- (18) deCANDOLLE, A. (1964).
Origin of Cultivated Plants. 2nd edition PP. 168-312. Hafner Publishing Company, New York.

- (19) De CARNERI, I. and Vita, G. (1973).
Drugs used in cestode Diseases, In:
Carvier, R. (Editor), Chemotherapy of
Helminthiasis Vol. I.
PP. 145-213.
Pergamon Press, Oxford.
- (20) CARTER, S.J. (1975).
Dispensing for Pharmaceutical Students.
PP. 120-167.
Pitman Press, Bath, England.
- (21) CARVIER, R. (1973).
Chemotherapy of Helminthiasis Vol. I:
PP. xi-xiii; 215-436.
Pergamon Press, Oxford.
- (22) CARVIER, R. and Lahmani, R. (1963).
Contribution à l'étude d'une méthode
d'essai in vivo des antiankylostomiens,
Annales Pharmaceutiques Françaises
21: 239-244.
- (23) CHAN, H.T. Jr. Heu, R.A. Tang, C.S.
Okazaki, E. and Ishizaki, S.M. (1978).
Composition of Papaya seeds. Journal of
Food Science 43: 255-256.
- (24) CHANDLER, W.H. (1964).
Evergreen Orchards.
2nd Edition. PP. 292-310.
Henry Kimpton, London.
- (25) CHOPRA, R.N., Chopra, I.C., Handa, K.L. and
Kapur, D.L. (1958). Indigenous Drugs of
India 2nd Edition. PP. 138
Dhur and Sons Private Ltd. Calcutta, India.
- (26) CHUTKO, M.B., Ryshkina, E.S. Pavlova, E.L. and
Yandiev, I.M. (1972). Opyt premeneniya
papaina v oftal' mologii (Experience using
papain in ophthalmology)
OFTAL' MOL 26(7): 542-543.
(Biological Abstract, 55(1): Abstr. 4212
(1973)).
- (27) COKE, J.L. and Rice, W.Y. Jr. (1965).
The Absolute Configuration of Carpaine.
Journal of Organic Chemistry, 30: 3420-
- (28) CSAPO, A. (1959).
Function and Regulation of the myometrium.
Annals of New York Academy of Science, 75:
790-808.

- (29) DAR, R.N., Garg, L.C. and Pathak, R.D. (1965).
Anthelmintic activity of Carica papaya
seeds. Indian Journal of Pharmacology,
27(12): 335.
- (30) DAS, P.N. and Bhakuria, B.N. (1974).
Anthelmintic effect of garlic (Allium
Sativum) against Ascaridia galli.
Vetcol Assam 14: 47-52.
(Helminthological Abstract, 44, Abstr.
1058 (1977).
- (31) DEVI, S. and Singh, S. (1978).
Changes in Placenta of Rat Fetus Induced
by Maternal Administration of Papain.
Indian Journal of Experimental Biology
16: 1256-1260.
- (32) EBASHI, S. (1961).
Calcium binding activity of vesicular
relaxing factor.
Journal of Biochemistry, Tokyo 50: 236-
244.
- (33) ELLINGER, M.G. and Hodgkins, J.E. (1956).
The mustard Oil of Papaya seed.
Journal of Organic Chemistry 21: 204-206.
- (34) ELLINGER, M.G. and Kjaer, A. (1968).
Recent Advances in Phytochemistry In:
Mabry, T.J., Alston, R.E., and Runeckles
(Editors), Vol.I:
PP. 125.
Appleton-Century, Croft, New York.
- (35) EL-TAYEB, O., Kucerova, M., Marquis, V.O. and
Kučera, H. (1974). Study on Carica papaya
seeds as a source of a reliable antibiotic,
the BITC. Planta Medica 26(1): 79-89.
- (36) EMELE, J.F. Shanaman, J. and Winbury, M.M.
(1966). The analgesic-anti-inflammatory
activity of Papain. Archives internationales
de Pharmacodynamic et de therapie, 159(1):
126-134.
- (37) FAIRCHILD, D.G. (1943).
Garden Islands of the Great East. C.
Scribner's Sons, New York.
- (38) FINBERG, J. (1960).
Smoking compositions. Delivered to
ERB-Tobacco Products Company U.S.A
(Chemical Abstract, 54; Abstr. 15856 d(1960).

- (39) FLATH, A.R. and Forrey, R.R. (1977).
Volatile components of Papaya (Carica papaya L. Solo variety). Journal of Agricultural and Food Chemistry. 25(1): 103-109.
- (40) GILES, R.E., Finkel, M.P. and Leeds, R. (1970).
The production of an emphysema-like condition in rats by the administration of papain aerosol (34750). Proceedings of the society for Experimental Medicine and Biology. 134: 483.
- (41) GINSBORG, B.Z. and Warriner, J. (1960).
Isolated chick biventer cervicis nerve-muscle preparation. British Journal of Pharmacology and Chemotherapy, 15: 410-411.
- (42) GOPALAKRISHMAN, M. and Rajasekharasetty, M.R. (1978).
Effects of Papaya (Carica papaya Linn) on pregnancy and oestrous cycle in albino rats of Wistar Strain. Indian Journal of Physiology and Pharmacology, 22(1): 66-70.
- (43) GOVINDACHARI, T.R., Pai, B.R. and Narasimhan, N.S. (1954).
Pseudocarpaine, a new alkaloids from Carica papaya L. Journal of chemical society, 1954: 1847.
- (44) GRESHOFF, M. (1890).
Isolation of carpaine from Carica papaya. Mededeel Uit'S Lands. Plant Buitenzorg, 7: 5-11.
- (45) GROSS, P. Babyak, M.A., Tolker, E. and Kaschak, M. (1964).
Enzymatically produced emphysema. Journal of Occupational Medicine, 6: 483.
- (46) GWATKIN, R.B.L. (1964).
Effects of enzymes and acidity on the zona pellucida of the mouse egg, before and after fertilization. Journal of Reproduction and Fertility 7: 99.
- (47) HÄGERMARK, O. (1973).
Influence of Antihistamines, sedatives, and Aspirin on experimental itch. Acta Dermatovener (Stockholm). 53: 365-368.

- (48) HEAD, W.F., Jr. and Lauter, W.M. (1956).
Phytochemical Examination of the leaves
of Carica papaya L. Economy Botany 10: 253-2
- (49) HEID, J.L. and Curl, A.L. (1944).
Papaya Products. Food Production. Journal
of American Food Manufacture, 24(2): 41.
- (50) HILL, A.F. (1937).
Tropical Fruits. Economic Botany
PP. 455.
McGraw-Hill Company, New York.
- (51) HORNICK, C.A., Sanders, Z.I. and Lin, Y.C.
(1978). Effects of carpaine, a papaya
alkaloid on the circulatory function on
the rat. Research communication in
chemical Pathology and Pharmacology 22:
2771-289.
- (52) HUXLEY, A.F. (1964).
Muscle, Annual Review of Physiology,
26: 131-152.
- (53) HUXLEY, H.E. (1965).
Structural evidence concerning the
mechanism of contraction in striated
muscle. In: Paul, W.M., Daniel, E.E.,
Kay, C.M. and Monckton, G. Muscle.
PP. 3-28.
Pergamon Press New York.
- (54) IBRAHIM, M.A., Nwude, N., Aliu, Y.O. and
Ogunsisi, R.A. (1983). Traditional
Concepts of Disease and Treatment among
Fulani herdsmen in Kaduna State of
Nigeria; In Press.
- (55) JOHNSON, W.G., Pierce, A.K. and Reynolds, R.C.
(1971). The evolution of papain emphysema
in the rat. Journal of Laboratory and
Medicine 78: 599.
- (56) JONES, W.W., Storey, W.B. Parri, G.K. and
Holdaway, F.G. (1940). Papaya production
in the Hawaiian Islands.
Hawaii Agricultural Experiment Station,
Bulletin 87.
- (57) KAIGHN, M.E. (1964).
A biochemical study of the hatching process
in fundulus heterochitus, Developmental
Biology. 9: 56.

- (58) KATAGUE, D.B. (1964):
Unpublished Ph.D. Thesis, University of Illinois.
- (59) KATAGUE, D.B. and Kirch, E.R. (1965).
Chromatographic Analysis of Volatile components of Papaya Fruits.
Journal of Pharmaceutical Sciences,
54: 891-894.
- (60) KATIYAR, J.C. and Sen, A.B. (1971).
Chemotherapeutic Properties of some New Isothiocyanates: Their cestocidal Action against Hymenolepis nana
Indian Journal of Medical Research
59: 949-956.
- (61) KLAUS, N. and Lee, K.S. (1969).
Influence of cardiac glycosides on calcium binding in muscle subcellular components.
Journal of Pharmacology, 166: 68.
- (62) KRISHNAKUMARI, M.K. and Majumder, S.K. (1960).
Studies of Anthelmintic activity of seeds of Carica papaya Linn. Annals of Biochemistry and Experimental Medicine,
20: 551-556.
- (63) Lal, J., Chandra, S., Raviprakash, V. and Sabir, M. (1976). In vitro Anthelmintic action of some indigenous medicinal plants on Ascarida galli worms. Indian Journal of physiology and pharmacology, 20(2): 64-67.
- (64) LEE, K.S. Hong, Sa. A. and Kang, D.H. (1970).
Effect of Cardiac glycosides interaction calcium with mitochondria.
Journal of Pharmacology 172: 180.
- (65) LINDLEY, J. and Moore, T. (1876).
The Treasure of Botany Rev. Edition
PP. 223-224.
Longman, Green and Company, London.
- (66) LODER, J.S. (1973).
Treatment of Jelly fish stings.
Journal of the American Medical Association.
226: 1228.
- (67) MEHTA, R.K. and Parashar, G.C. (1966).
Effects of Butea frondosa, Vernonia anthelmintica and Carica papaya against Oxyrids in mice
Indian Veterinary Journal 43: 744-749
- (68) MILNE, J. and Brand, S. (1975). Occupational

asthma after inhalation of dust of the proteolytic enzyme papain. British Journal of Industrial Medicine, 32: 302.

- (69) MODI, N.J. (1969).
Mod's Textbook of Medical Jurisprudence and Toxicology
 PP. 45.
 N.M. Tipathi Private, Bambay, India.
- (70) NAGATY, H.F., Rifaat, M.A. and Morsy, T.A. (1959). Trials of the effects of dog Ascaris in vivo produced by latex of Carica ficus and Carica papaya growing in Cairo gardens. Annals of Tropical Medicines and Parasitology 53: 215-219.
- (71) NEEDHAM, D.M. and Shoenberg, C.F. (1967). The Biochemistry of the myometrium. In: Wynn, R.M. (Editor) Cellular Biology of the uterus. PP. 291-352. Appleton-Century Crofts, New York.
- (72) NELSON, A. (1951).
Medical Botany, PP. 301.
E & S Livingstone Ltd. Britan.
- (73) NNOCHIRI, E. (1975).
Medical Parasitology in the Tropics
 PP. 5-15.
Oxford University Press, London.
- (74) NOBLE, I.G. (1946).
 Fruta bomba (Carica papaya) in hypertension, Anales acad. Cienc. Med. fis y nat. Habana 85: 198-203.
 (Chemical Abstract, 45: Abst. 3519⁷ (1951)).
- (75) NOVEY, H.S., Marchioli, L.E., Sokol, W.N. and Wells, I.D. (1979). Papain-induced asthma-Physiological and immunological features. Journal of Allergy and clinical immunology 63: (2): 98-103.
- (76) OGAN, A.V. (1971).
 The basic constituents of the leaves of Carica papaya. Phytochemistry 10: 2544-2547.
- (77) OLIVEROS-BALARDO, L., Masilungan, V.A., Cardeno, U., Luna, L., de Vera F., la Cruz, E. and Valmonte (1970). Possible anti-tumor constituent of Carica papaya L. Paper presented at Scientific Session, "Third Asian Congress of Pharmaceutical Sciences" held at Manila, Philippines, 16-20 November.

- (78) ONUAGULUCHI, G. (1964).
Anti-ascaris activity of certain extracts
from the bark of *Polydora umbellata*
(Daziel) (Erin-Yoruba).
West African Medical Journal 13(4): 162-165.
- (79) OSGOOD, H. (1945).
Atopic sensitivity of caroid (papain)
Journal of Allergy, 16: 245.
- (80) POUSSET, J.L., Boum, B. and Cavé, A. (1981).
Action Antihémolytique du xylitol Isole
des Ecores de *Carica papaya*
Planta Medica 41: 40-47.
- (81) PURI, G.S. and Talalaj, D. (1964).
A survey of some plants used in native
medicine in West Africa, of interest to
India. Paper presented to the symposium
on "Recent advances in the development,
production and utilization of medicinal
and aromatic plants in India", held at
Jammu (Kashmir), India, 3-5 December.
- (82) PUSHPAKOM, R. Hogg, J.C. Woolcock, A.J. Angus,
A.E. Macklem, P.T. and Thurlbeck, W.M.
(1970). Experimental Papain-induced
emphysema in dogs. American Review of
Respiratory Disease. 102: 778.
- (83) RAMSAWAMY, A.M. and Sirsi, M. (1960).
Antituberculosis Activity of some chemical
constituents from Higher Plants.
Indian Journal of Pharmacology, 22: 34-35.
- (84) ROBINSON, G.W. (1975).
Isolation and characterization of Papaya
peptidase A from commercial chympapain.
Biochemistry. 14(16): 3695-3700.
- (85) ROBINSON, P. (1958).
Seeds of *Carica papaya* for mass treatment
against Ascariasis.
Indian Journal of Child Health 7: 815-817.
- (86) ROLLO, I.M. (1980).
Drugs used in the chemotherapy of
Helminthiasis In. Gilman, A.G., Goodman,
L.S. and Gilman, A. (Editors). The
Pharmacological Basis of Therapeutics.
Macmillan Publishing Company, New York.
PP. 1061-1969.

- (87) SEN, H.G., Joshi, B.S. Parthasarathy, P.C. Kamat, V.N. (1974). Anthelmintic efficacy of Diospyrol and derivatives Arzneimittel-Forschung 24(12): 2000-2003. (Helminthological Abstract, 44(11): Abstr. 5551 (1975).
- (88) SCHACK, P. (1967). "Fractionation of proteolytic enzymes of dried papaya latex. Isolation and preliminary characterization of a new proteolytic enzyme. Comptes Rendus des Travaux du Laboratoire Carlsberg 36(4): 67-83. (Biological Abstract, 48: Abst. 113684 (1967)).
- (89) SHAPIRA, E., Giladi, A. and Neuman, Z. (1973). Use of water insoluble papain (WIP) for debridement of burn eschar and necrotic tissue. Plastic and Reconstructive Surgery 52: 279.
- (90) SHOENBERG, C.F. (1969). An electron microscopic study of the influence of divalent ions on myosin filament formation in chicken gizzaro extracts and homogenates, Tissue and Cell 1: 83-96.
- (91) SINGH, S. and Devi, S. (1978). Teratogenic and emryotoxic effect of papain in rat. Indian Journal of Medical Research. 67: 499-510.
- (92) SMALBERG, T.M. Rall, G.J.H. and De Waal (1968). Tydskrif vir Natuurweten skappe (Neth.) 8 (3-4): 156-157. (Chemical Abstract, 71: Abstr. 36405v, (1969).
- (93) SMITH, D.G. (1964). Techniques in enzyme hydrolysis, In: Hirs, C.H.W. Methoth in Enzymology XI, Enzyme Structure PP. 214 Academia Press, New York.
- (94) SOFOWORA, A. (1982). Medicinal Plants and Traditional Medicine In Africa. PP. 44, 68. John Wiley and Sons Limited, Chichester, U.S.A.

- (95) SPITELLER-FRIEDMAN, M. and Spiteller, G. (1964).
Application of Mass Spectrometry To The
Elucidation of Alkaloid V. The Structure
of Carpaine.
Montash. Chem. 95; 1234-1241.
(Chemical Abstracts, 62: Abstr. 4072g
(1965).
- (96) STANDEN, O.D. (1963).
Chemotherapy of helminthic infections.
In: Schnitzer, R.J., and Hawking, F.
(Editors),
Experimental Chemotherapy, Vo. I.
PP. 701-892.
Academic Press, New York.
- (97) STARKOV, G.L. and Savinykh, V.I. (1971).
Ekstraktsiya Oslozhennykh Katarakt
(Extraction of complicated adherent
cataracts). OFTAL' MOL ZH 21(1):
15-18. (Biological Abstract, 54(9):
Abstr. 52048 (1972).
- (98) STARKOV, G.L. Saprykin, I.D. and Savinykh, V.I.
(1970). Lechenie Papainom Zabolevanii
rigovoi Obolochki (Diseases of the cornea
treated with papain).
OFTAL' MOL ZH 25(5): 351-354.
(Biological Abstracts, 52(10): Abstr.
56797 (1971).
- (99) STOLL, M.A. (1937).
This wormy world. Journal of Parasitology
33: 1-18.
- (100) STOREY, W.B. (1969).
Papaya, In: Ferwerda, F.P. and Wit, F.
Outlines of Perennial Crop breeding in the
Tropics. PP. 389-407.
H. Veeman and Zonen, N.V., Wageningen,
The Netherlands.
- (101) TANG, C.S. (1971).
Benzylisothiocyanate of papaya fruit.
Phytochemistry 10: 117-121.
- (102) TANG, C.S. (1973).
Localization of Benzyl glucosinolate
and Thioglucosidase in Carica papaya.
Phytochemistry 12: 769-773.

- (103) TANG, C.S. Bhothiapaska, K. and Frank, H.A. (1972). Bacterial Degradation of Benzylisothiocyanate. Applied Microbiology, 23(6): 1145-1148.
- (104) TARLO, S.M., Shaikh, W., Bell, B., Curf, M., Davies, G.M., Dolovich, J. and Hargreave, F.E. (1978). Papain induced allergic reactions. Clinical Allergy 8: 207-215.
- (105) TO, S. and Kyu, C. (1934). Amoebicidal Activity of Alkaloids, Journal of Medical Science, 8: 52-54.
- (106) TUFFLEY, B.J.M. and Williams, C.H. (1951). The Pharmacology of Carpaine. Australian Journal of Pharmacology 52: 796-798.
- (107) VOGEL, A.I. (1974). Practical Organic Chemistry, including Qualitative Organic analysis. PP. 1079. Longman Group Limited, London.
- (108) WATT, G. (1972). In: Dictionary of Economic products of India. Vol. II 2nd Edition., PP. 57-58. Cosmos Publications, Delhi, India.

APPENDIX

Concentration of solutes in grams per litre
in Kreb's Solution, NaCl = 6.9, KCl = 0.35, MgSO₄.
7H₂O = 0.29, CaCl₂ = 0.28, KH₂PO₄ = 0.16
NaHCO₃ = 2.1 and D-Glucose = 2.0.

Concentration of Solutes in grams per litre
in De Jalon Solution, NaCl = 9, KCl = 0.14,
D-Glucose = 0.5, NaHCO₃ = 0.5 and CaCl₂ = 0.03.

Concentration of Solutes in grams per litre in
Ringer-Lock Solution, NaCl = 9, KCl = 0.42, D-Glucose = 1,
NaHCO₃ = 0.15 and CaCl₂ = 0.12