PHARMACOLOGICAL RATIONALE FOR FOLKLORIC USES OF THE LEAVE EUPHORBIA HIRTA, LINN

By

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A THESIS SUBMITTEDTHE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENT DEGREE OF MASTER SCIENCE IN PHARMACOLOGY

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Ahmadu Bello University, Zaria.

NOVEMBER, 1992.

Kasco Scientific Agency, Jos.

DECLARATION

I carried out this work under the supervision of Dr. I.M. Hussaini and Dr. I. Abdu-Aguye of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The work of other investigators are duly acknowledged and referred to accordingly.

I hereby, declare that no part of the work reported in this thesis, has been submitted elsewhere for a degree.

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DEDICATION

To my parents
Amy** and **Francis Johnson

all the brethren of the Postgraduate Fellowship and the Fellowship of Christian Students, A.B.U., Zaria, who have cared in one way or the other. Many thanks to Mr. O.O. Adeluyi, Bayo & Shola of Kasco Scientific Agency, Jos and J. Nnachi - who typed this thesis. God bless you richly.

To the One who WAS, IS, and IS TO COME - the Lord Jesus, be all glory, honour and praise. Words cannot express my thanks Father, but I pray you will be glorified in my life. Amen!

ABSTRACT

Water and ethanol extracts of the leaves of Euphorbia hirta were subjected to in vivo and in vitro studies. Water extract (0.025 - 2.5 mg/ml), ethanol extract (0.25 - 1 mg/ml); fraction 28 of ethanol extract (0.1 - 0.5 mg/ml) and adrenaline (2.5 - 250 ng/ml) produced concentration - dependent relaxations of the rabbit jejunum. Ethanol extract also caused concentration -related contractions of the same tissue. The relaxations produced by the extracts and adrenaline were not affected by atropine (10 nM) but blocked by propranolol (1 /xM). The extracts and fraction 28 have shown bacteriostatic rather than bactericidal effect.

Low concentrations of water extract (10 - 100 µg/ml) and ethanol extract (0.05-5 mg/ml) had no observable aggregatory effect on human platelets. However, PAF (50 - 1OOOnM) and adrenaline (1-10 µg/ml) caused a concentration-related aggregation of human platelets. Both extracts had no observable effects on aggregation induced by PAF and adrenaline. Water extract (0.2 - 1 mg/ml) produced concentration-related aggregation of human platelets maintained in plasma. Indomethacin (0.014 - 0.14 µM) produced a concentration-related inhibition of water extract-induced platelet aggregation and this was also reduced by nifedipine (0.23/µM) whilst WEB 2086 had no observable effect.

The water extract (0.05 - 5 mg/kg) and the ethanol extract (0.5 - lmg/kg) produced dose-dependent decrease in the mean arterial blood pressure (MABP) of the cat. These effects were partially blocked by atropine (5.0 and 10 µg/kg) and pirenzepine (5 mg/kg) while propranolol (1 µM/kg) had no effect. Further investigation of the mechanism using methroctramine (0.6 µM) caused a blockade of water (0.5 mg/ml), acetylcholine (1 µg/ml) and choline (0.5 mg/ml) which had earlier produced maximum **relaxation** on **the guinea pig** atrium.

The diuretic effects of the extracts were assessed using acetazolamide and frusemide as standard drugs. Water extract (50 and 100 mg/kg) and ethanol extract (50 and 100 mg/kg) produced time-related and significant increase in urinary output. Electrolyte excretion was also significant especially for bicarbonate ions (HCO_3''). The water extract and acetazolamide have similar spectrum of diuresis.

The acute toxicity test was carried out to determine the safety margin of the water and ethanol extracts of Euphorbia hirta. The LD_{50} values were 1.13 ± 0.21 for water extract and 1.61 ± 0.24 g/kg for ethanol extract. These values fall within the slightly toxic range. The mechanism of E.hirta induced-death supports the presence of compounds (eg. leukotrienes) which cause respiratory distress.

These studies on E.hirta have provided data supporting the folkloric use of the plant in the treatment of diarrhoea and possibly dysentery, and also as a diuretic remedy. However, no pharmacological basis was found for the traditional use of the plant as an anti-asthmatic agent. This was further supported by the acute toxicity test. Pharmacological screening revealed the presence of a hypotensive principle(s).

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ABBREVIATIONS AND SYMBOLS

Ach	Acetylcholine
Adr	Adrenaline
Cl-	Chlorine ion
cm	centimeter
CO_2	Carbondioxide
EC_{50}	effective concentration for 50% response
EtOH	ethanol
g	gram(me)
h	hour(s)
HCO_3-	bicarbonate ion
H_20	water
i.p.	intraperitoneal
i.v.	intravenous
K^+	potassium ion
kg	kilogram
MABP	Mean Arterial Blood Pressure
mg/kg	milligram per kilogram
mg/ml	milligram per millimetre
min	minutes
mmHg	millimeter of mercury
NDGA	norhydroguairetic acid
ng	nanogram
O_2	oxygen
%	percentage
PRP	Platelet rich plasma
S.E.M.	standard error of the mean
U/kg	unit per kilogram
w/v	weight per volume
μM	micromole

GLOSSARY OF MEDICAL AND OPERATIONAL TERMS

Aphrodisiac	-	An agent which stimulates sexual excitement.
Bactericide	-	Agent which destroys bacteria.
Bacteriostatic	-	Agent that arrests or hinders bacterial growth.
Cascade	-	Stepwise linkage.
Cathartic	-	Producing bowel movements.
Coma	-	Complete loss of consciousness.
Defaecation	-	Voiding of faeces
Diarrhoea	-	Irregular bowel rhythm characterized by an increase in frequency and fluidity of the stools.
Dislocation	-	A displacement of organs or articular surfaces so that all apposition between them is lost.
Diuretic	-	An agent which increases the flow of urine
Dysentery	-	Diarrhoea with blood and mucous, together with pain and tenesmus (unproductive straining)
Exsanguination	-	The process of rendering bloodless.
Folkloric	-	Describes the customs and beliefs of a people.
Lethal	-	To cause death.
Micturition	-	The act of passing urine.
Palsy	-	Paralysis
Powder	-	Mass of fine particles
Pretreatment	-	Prior administration of a drug or agent to check effect on another drug.
Prostration	-	Lying flat with face downwards.

CHAPTER ONE

1.1 GENERAL INTRODUCTION

A drug is a chemical compound incorporated into a medicine, that has either curative, prophylactic or diagnostic effects on the body. Drugs are obtained from two sources (ie. The Natural source which are from plants, animals and minerals; and the Synthetic source which are those chemically synthesized). However, irrespective of the source, a drug is meant to perform the earlier stated purpose of cure, prophylaxis or diagnosis, and thus keep the individual in top form and good health.

However, since the earliest times, the Plant Kingdom in particular, has proven to be most useful for the treatment of various ailments. In developing countries, especially in Africa and Australia, plant derived drugs or herbals have formed the mainstay of traditional remedies.

The continued popularity of traditional remedies is enhanced by the ready availability of plant materials when compared to modern drugs in rural areas and less accessible regions in developing countries, shortage of practitioners of modern medicine and the safety of such preparations. According to Kordel (1976) "Herbs never cure one disease by creating another, as most synthetic drugs do. They are slower in action, but safer; for no risk is involved in their long-term use." This is however debatable.

In most developing countries, tradition represents a way of life, along with this comes the use of herbal remedies. As such, people in the rural areas tend to believe more in the traditional medicinal practitioner than in "orthodox" practitioner, for the former is part-and-parcel of the community. Whereas, the "orthodox" practitioner is a product of modern medicine which is looked on with suspicion, as an alien system imposed on the

people. Since independence, many countries have sought ways of incorporating the traditional system of healing into orthodox medicine; organizations like the Organization of African Unity/Scientific Technical Research Coorporation (OAU/STRC) have been actively involved in trying to make this aim a reality.

1.1.1 APPROACHES TO PLANT DERIVED DRUG DEVELOPMENT

Preliminary to any pharmacological evaluation, one has to decide which plants to collect out of the several species available. At present, no scientific methods exist for the selection of plants expected to have biologically active substance. Thus, four "non-scientific" methods have been used over the years in the course of such research. These are:

i) Phytochemical screening.

ii) Random selection approach.

iii) Selection of plants containing specific types of chemical compounds.

iv) Folkloric procedure.

The most successful of these methods is the selection of plants based on folkloric use (Farnsworth, 1984).

Folkloric procedure

The scientific investigation of many plants claimed to have medicinal properties from folkloric use, has led to the discovery of many useful drugs some of which are useful investigative tools in pharmacological studies. The discovery of several alkaloids, for example, reserpine (from Rauwolfia serpentina) used in hypertension; tubocurarine, a muscle relaxant from the arrow and dart poison - curare; the alkaloid, physostigmine; a cholinesterase inhibitor from Physostigma venenosum and a host of others like digitoxin and

digoxin; which are cardiac glycosides from Digitalis purpurea; morphine and codeine from Opium species are just a few of the useful drugs derived from plants through this procedure (Farnsworth, 1984, Phillipson et al, 1989).

1.1.2 **The contribution of Medicinal plants to Modern medicine**

Although the use of plants for medicinal purposes may be considered to be mainly within developing countries, there can be no doubt that there is an increasing swing towards herbal medicines in Western countries (Phillipson, 1981). Consequently, there has never been such a time in our history, that the need for ethnopharmacological studies are so essential.

The stated aim of the World Health Organisation (WHO) to provide "Health for all by the year 2000" has helped to focus attention on traditional medicine, which is used extensively in all countries in the developing world (Bannerman et al, 1988). Since traditional medicine was incorporated into WHO's programme in 1976; there has been more co-operation between practitioners of orthodox and of traditional medicines.

In the 18th and 19th centuries, pharmacologically active principles of a number of plants were isolated and it was realised that the clinical effects of drugs such as cinchona, opium, ipecacuanha could be attributed to the chemical compounds, quinine, morphine and emetine, respectively. These early isolated active principles were all alkaloids and though they could not replace the clinical activity of such plant, yet they were found to have substantial activity when administered in standardised dosage forms. Today, many plant-derived medicines are administered as purified, isolated compounds made into appropriate pharmaceutical dosage forms.

Although examples abound from many different types of natural products, the alkaloids, in particular have proven to have a wide range of pharmacological actions and clinical applications. A few other groups are cardiac glycosides e.g. digoxin, digitoxin and lanatoside; anthraquinone laxatives e.g. danthron, aminoglycosides e.g. streptomycin, neomycin; carbohydrates e.g. starch, sucrose.

The alkaloid, physostigmine isolated from Calabar beans - Physostigma venenosum, was shown to inhibit acetylcholinesterase and thus has an opposite pharmacological effect to atropine (which is a solanaceous alkaloid that blocks muscarinic receptors on which acetylcholine acts). Once the chemical and pharmacological studies established the structure for activity of Physostigma venenosum, investigations led to the synthesis of neostigmine, which is used mainly for the treatment of myasthenia gravis, a disease in which there is impairment of neuromuscular transmission (Phillipson et al, 1989). Atropine was obtained through the same process; and this led to the synthesis of other compounds like homatropine which has enhanced and specific activity.

The South American arrow and dart poison-curare, which was used to paralyse hunted animals, blocks neuromuscular transmission. The active principle, tubocurarine was isolated from it. Using this molecule as a template, a range of neuromuscular - blocking agents have been brought into clinical practice as muscle relaxants. Such compounds e.g. Metocurine (ie Dimethyltubocurarine) have been proven to be particularly useful in surgery, where muscle relaxation is of utmost importance (Phillipson et al, 1989).

Plants and plant products are to be found in preparations recommended for the treatment of fourteen out of the fifteen therapeutic categories (Prased, 1987). The types of natural product ingredient include alkaloids, balsams, carbohydrates, cardenolides, coumarins, enzymes, essential oils, fixed oils, flavonoids, resins, steroids, triterpenes and

vitamins. The use of many of these materials as pharmaceuticals has resulted from ethnopharmacological observations. Although the contribution of these plant-derived substances to the therapeutic categories is considerable, it is even more so when further factors are considered; e.g. the use of coumarin derivatives as anticoagulants, bromocriptine for the treatment of infertility and prevention of lactation, and the cannaboid derivatives as anti-emetics, among many others.

Thus, without plants, the majority of chemicals would not be available for use and currently scientific literature research into the chemistry and pharmacology of biologically active natural products is advancing at a fast rate. The techniques available for scientific evaluation of biologically active compounds are becoming increasingly more sensitive and many more in vitro tests are now available for primary screening of extracts without resorting to whole animal experiments (ie. in vivo tests) (Holmstedt et al, 1983).

1.1.3 Collection of data on the use of medicinal plants

There exists a vast amount of literature which can serve as a source of information on medicinal plants. However, since this literature is not readily available, computerized database search has become indispensable. This method is however disadvantageous in that the exact use of the medicinal plants is not described in a very detailed form, neither dose, dosage forms, nor the kind of diseases treated is reported in any detail (Verpoorte, 1989). However it provides information concerning where such details can be obtained, therefore further literature search is needed.

Furthermore, translation of ideas from traditional medicine into modern medicine creates problems in the evaluation of traditional remedies. Therefore field studies of the use of these traditionally useful plants is an important additional tool (Verpoorte, 1989). Such

studies will include enquiry made in villages on plants found in the vicinity or herbarium specimens, consultation of a herbalist, consultation of a herb-seller; attending a course on herbalism; information from folk-tales and sending volunteer patients for herbal treatment with subsequent checks on prescription (Sofowora, 1984).

The use of traditional medicines by herbalists is often connected with some kind of magic or mysterious supernatural power. The separation of fact from fiction will be a major task for the researcher in field studies. The herbalist may specify that a plant must be collected at night, or even at a specific time of night e.g. full moon, for it to have its desired efficacy. The time of collection may easily be regarded as magic. However, considering that most plants have a day-night rhythm and secondary metabolism might be different in this diurnal cycle, the importance of the time of collection could be explained (Verpoorte, 1989). Waller et al., (1978) reviewed some illustrative examples: Papaver sommniferum showed a diurnal variation in alkaloid content; codeine being at its maximum (300% above the daily average) at noon (daily variation from 10 - 300%) and morphine at its maximum of almost 200% from 8 - 10 a.m (daily variation from 50 - 200%).

The status of the plant is also an important factor in field studies. Flowering could for example, be an obvious point of difference in the secondary metabolism.

1.1.4 **Screening for biological activity**

a) Plant collection and identification

In African traditional medicine, it is common for the same plant to bear several different local names. This more than often leads to the collection of the wrong plant. Therefore in all screening work, identification of the plant, must be confirmed to avoid confusion in the future and to enable research workers in the field to confirm

the findings. As a result of various symposia held on problems in discovery of new drugs with specific attention to those posed by incorrect nomenclature, some international, scientific and research organizations like WHO, OAU/STRC and United Nations Educational Scientific and Cultural Organization (UNESCO) decided that a specimen of any plant investigated should be deposited in a recognized herbarium, after identification has been confirmed (Sofowora, 1984). Infact, many reputable, scientific journals on natural products and phytochemistry research would only consider an article for publication, after the specimen has been identified and deposited in a reputable herbarium of known address.

b) Extraction procedure

The next step after identification is the choice of extraction procedure. This is based on the use of different solvents with varying polarities to yield varied extracts with different constituents. The method used determines the type of compounds that will be present. (Verpoorte, 1989).

c) Screening

The selected, documented and extracted plant or plant part is now ready for evaluation of the biological activity. The design of this evaluation is based on the aims of the study, either a specific assay for a certain activity or a general screening to evaluate all possible activity.

The Hipprocratic screening has been used in time past, and it has been advocated as an excellent method for screening (Malone et al, 1962; Malone, 1977). However, it requires large amounts of extracts and is therefore not very suitable for guidance on the isolation of

an active component (Verpoorte, 1989). In the preliminary biological screening of plant extracts, blind and phytochemical screening were the procedures first considered (Lockwood, 1979). The purpose of blind screening is to discover whether the extract has any activity and in which area of the pharmacological spectrum this lies. Thus an inactive extract will be discarded (Sofowora, 1984).

The screening is divided into three categories namely, the primary, secondary and tertiary levels. The primary screening involves in vitro studies (carried out on isolated tissues) and in vivo studies (whole animal studies). The in vitro studies are carried out first because they are cheaper, quicker and generally more reproducible. The secondary and tertiary screening involve a logical succession of specific tests - selection of which are based on the previous results from the primary screening. They are advanced pharmacological and toxicological studies.

Basic phytochemical screening consists of performing simple chemical tests to detect the presence of specific chemical groups e.g. alkaloids, tannins, saponins, anthraquinones, cardenolides, glycoside, flavonins, etc. in a plant extract. Standard chemical tests have been devised for these screening which are simple and rapid as many samples would have to be handled (Sofowora, 1984).

1.2 LITERATURE REVIEW

Euphorbia hirta (Linn)

1.2.1 Taxonomy

Kingdom	-	Plantae
Phyllum	-	Angiosperm
Class	-	Magnoliopsida

Subclass	-	Rosidae
Order	-	Euphorbiales
Family	-	Euphorbiaceae
Genus	-	Euphorbia
Species	-	hirta

1.2.2 EUPHORBIACEAE

This is also known as the Spurge family. This is a large family of cotton-like trees and shrubs which often yield a milky juice (latex) when a plant part is damaged. They occur in many tropical and temperate regions, and a few herbaceous species exist in cooler regions. Many of the tropical species are interesting xerophytes (Considine, 1976).

The leaves are alternate and have stipules, they are obliquely ovate to lanceolate, rounded on one side; cuneate on the other side, at the base, acute or subobtuse at the apex. They could be up to 5 cm long and 2 cm broad. They are finely serrated and thinly pilose. The cymes are mostly pedunculate (Hutchinson et al, 1928; Hutchinson et al, 1958). The inflorescence in members of this family is often very complex. In some species, the individual flowers are crowded together in such a way as collectively to resemble a single large flower. The flowers are unisexual. The plants are either monoecious or dioecious. In many cases, the flowers entirely lack both calyx and corolla; in others a calyx is present, but no corolla; while in some, both calyx and corolla are present. They are regular flowers with the perianth commonly 5-parted. The number of stamen varies, they could be either joint or branched. The ovary and capsule are hairy. The fruit is about 1.5 mm broad. The ovary is usually 3-celled with one or two ovules in each cell. The fruit is a capsule with seeds containing an abundant endosperm and a caruncle (Considine, 1976). Involucres are

borne in dense, rather long, pedunculate axillary and terminal leafless glomerulus. The stem and branchlets have rather coarsely spreading pilose with yellowish hairs over a short indumentum (Hutchinson et al., 1928; Hutchinson et al., 1958).

1.2.3 EUPHORBIA

Euphorbia hirta (Linn) is a species in the family - Euphorbiaceae. The herb is an annual herb, indigenous to the hotter parts of India and occuring in all tropical countries. The stem is slender, erect or ascending, growing up to about 30 - 60 cm high. The young, upper parts are covered with yellow, spreading hairs and pubescent with short, curved hairs; the older parts have reddish-purple patches. The cylindrical stem contains a milky latex which exudes when the plant is damaged. The leaves are oppositely arranged, lanceolate and about 2-5cm long, they are greenish or reddish underneath assymetrical and of rounded base; in axils of which appear very small dense, round clusters of flowers. The apex is acuminate. The small green flowers constitute the inflorescence characteristic of the Euphorbias. The fruit is a minute, yellow three-celled capsule, about 1 mm in diameter each of the three carpels being distinctly keeled, and containing a single, reddish, four-sided transversely, wrinkled seed. These capsules are hairy. (Wallis, 1951; Lind et al, 1971; Kerharo et al, 1974).

In commerce, E.hirta is popularly known as Euphorbia pilulifera. It is commonly known as Australian asthma herb, Queensland asthma weed; Pillsbearing spurge; cat's hair; Hairy spurge; spurge or milkweed. Its local name in the northern part of Nigeria, where it is most popular, is "Nonon kurchiya" which means pigeon's milk due to the milky exudate; in Yoruba it is "egele" or "emi-ile" which means cassava in the ground or shea-butter tree in the ground. (Dalziel, 1948).

1.2.4 TRADITIONAL OR MEDICINAL USES OF E.HIRTA

Euphorbia hirta has had a long folkloric history of use for the treatment of various ailments. This plant has been used in almost every part of the world, particularly in rural Africa (Khan, 1980).

In East and West Africa, extracts of the decoction of the flowers and fruiting plants are used in the treatment of asthma and respiratory tract infections and it is sometimes combined with bronchial sedatives like Grindelia robusta in preparations for inhalation (Oliver, 1959; Kokwaro, 1976). However, some claim that it causes asthma (Hussaini, personal communication).

Its pectorial antispasmodic principles are used in the treatment of coughs, chronic bronchitis and other pulmonary disorders, especially in Australia (Wong-Ting Fook, 1980). It is also used for relieving hay-fever and catarrh of the head (Le Strange, 1977).

An infusion of the leaves has been used to increase lactation in sheep, goats and cattle in East Africa (Kokwaro, 1976) and it is used for the same purpose by Ghanian women. The women of Northern Nigeria also use it for the same purpose (Alhaji Alhassan Likoro, personal communication). The latex is used by young Malawian girls for greater breast development (Hargreaves, 1979). The plant is cooked with Bridelia pilosoe and eaten freely by women, to ease the tenderness associated with puerperium.

In Hawaii, the plant has been used as a cathartic gargle and poultice (Watt et al, 1962). The pounded leaves in water act as an enema for constipation. In Tropical and East Africa, the leaves and latex are used in the treatment of boils, sores and wounds especially ophthalmic wounds (Watt et al, 1962). The latex is also used for conjunctivitis and ulcerated cornea. It is used externally as an antidote to arrow poison and an antiseptic; the root is a snake-bite remedy in India (Watt et al, 1962).

An infusion of the plant is used in Angola and Portugal against diarrhoea and dysentery - due to its antimicrobial activity. The plant is also used by the Toukoulers and Wolofs for colic in infants (Watt et al, 1962; Sussman, 1980).

In Central African Republic, this plant is used as an aphrodisiac and as antihelmintic agent. (Dalziel, 1948; Oliver, 1959: Kerharo et al, 1974; Ayensu, 1978; Wong-Ting Fook, 1980). It is also a gonorrhoeal remedy, thus it is used to treat infections caused by gonococcus. In Northern Nigeria, it is used to treat some fungal infections like Tinea. The Sukumas also use it as a diuretic (Watt et al, 1962). Its antiparasitic activity against Entamoeba histolytica has been extensively utilized in the treatment of amoebic dysentery (Dhar et al, 1968; Oliver, 1983).

1.2.5 TRADITIONAL OR MEDICINAL USES OF OTHER RELATED SPECIES UNDER EUPHORBIACEAE

The family, Euphorbiaceae has been used in all types of ailments in most parts of rural Africa. Some of the other genera under these family are Alchornea, Antidesma, Bridelia, Croton, Erythrococca, Mecaranga and Manihot.

a) Alchornea cordifolia

The roots are used for treating jaundice, leprosy and snake bites. The pith of the roots are chewed for the treatment of thrush and buccal ulcerations. The bark is used for stomach-aches; an infusion of its shavings is used for treating diarrhoea. A decoction of leafy twigs is used to wash feverish chills, treat rheumatic pains and sores and is applied as a lotion or poultice to sore feet. This decoction is also used in combination with lime juice to cause purgation, and it is used as a wash for urethral discharge. The leaf decoction is used as an antispasmodic for headache, to treat coughs, colds and bronchial troubles,

eyewash for conjunctivitis, stomach ache, dysentery, menstrual pains and fever. The powdered leaves are used for treating gonorrhoea (Irvine, 1961).

b) Antidesma lacinatum

The powdered bark is soaked in water or palm wine and this decoction is taken three times a day as an aphrodisiac in Ivory Coast. In Liberia, bathing in the leaf decoction prevents abortion. (Irvine, 1961).

c) Bridelia ferruginea

The decoction of root is used as a diuretic and as a gonorrhoeal remedy. The bark and prickles are used as a mouthwash, and to treat thrush in children by the Yorubas. In Togo, the root bark is used for intestinal and bladder troubles. It is also used externally for skin diseases and eruptions. In Northern Nigeria, it is used as an antidote to arrow poison. The bark or root decoction (or in combination with cassava flour) is used to cure dysentery and diarrhoea in Ivory Coast. An infusion of the bark or leafy stems are used as a beverage and for vapour baths in case of fever, pains, headaches, stiffness and rheumatic pains (Irvine, 1961).

c) Erythrococca anomala

In the Futa Jallon area, the leaves are used as an antihelmintic specific for tapeworm. In Sierra Leone, the leaf is rolled to form a funnel and water is retained in it for sometime before allowing it to be applied to sore eyes. The powdered leaves and pepper are used as snuff for recurring headaches. The leaf pulp is used to massage intercostal pains, rheumatism and back-aches. The leaf juice is applied to the ear as drops in otitis. A

decoction of the leafy stems is used to wash wounds, ulcers and yaws abscesses. It is an antidote to snake bite (Irvine, 1961).

d) Euphorbia deightonii

A drop of the latex is administered as a purgative in man and livestock. It is also an ingredient of arrow poison and is usually applied to carious teeth to relieve ache and ease extraction. When mixed with grain, the latex is used to stupefy and ease the birth process. It is used for poisoning animals (Irvine, 1961).

e) Manihot esculenta

The raw, young roots of sweet cassava when steeped are lactogenic; thus it is used to increase lactation. When eaten with the leaves of Piper umbellatum, they allay the pains of pregnant women. The latex from the stem is used in parts of Ivory Coast as eyedrops for conjunctivitis. The crushed leaves are used to treat chicken pox and milk skin infections. The stewed and pulped leaves are applied to tumours in Nigeria (Irvine, 1961).

1.3 PHYTOCHEMISTRY

A preliminary screening of the whole plant revealed the presence of a given resin, calcium oxalate crystals, sugars, mucilage, volatile substances, melisyl palmitic, oleic and linoleic acids; traces of ceryl alcohol, an essential oil as well as malic and succinic acids (Kerharo et al, 1974). Ueda and Hsu (1949) isolated an alkaloid which was identified by then as xanthorhamin. This was shown to constitute about 2% (Watt et al, 1962). Blanc et al (1964) confirmed the presence of an alkaloid. In 1966, Gupta and Garg characterized from the stem extract, taraxerol, β-amyrin, friedelin and β- sitosterol. Ridet and Chartol

(1964) indicated that a saponin whose aglycone is rhamnose and a triterpenoid aglycone is present. Atallah and Nicholas (1972) identified taraxerol (in free form), taraxerone (esterified), α-and β-amyrin and β-sitosterol (70%) from the hot hexane extract of the leaves and stem of the plant.

Lewison found several glycosides, resins, wax and volatile matter. The dry herb has yielded gallic acid, a phenolic substance $C_{28}H_{18}0_{15}$, an alcohol- euphosterol $C_{25}H_{39}OH$ and jambulol (Power et al, 1913), Campesterol (20%), stigmasterol (10%) and sitosterol were found to occur both freely and in esterified forms (Atallah et al, 1972). Hallet and Parkes (1951) isolated i-inositol. The latex has been shown to contain irritant and cocarcinogenic phorbol esters e.g. euphorbon which is capable of producing dermatitis (Watt et al, 1962; Ayensu, 1979). The biogenesis of these esters seems to be affected by climatic variation (Upadhyay et al, 1980). The latices and seed oil of these plants have been shown to contain various polyfunctional, polycyclic diterpenes such as phorbol, ingenol and their derivatives (Upadhayet al, 1976; Kinghorn et al, 1975). Estrada (1959) showed that the stem and leaves are rich in flavonoids. Power and Browning (1913) isolated quercetin. This has been confirmed by Hallet et al, (1951) and by Blanc et al, (1972). This flavonoid is assumed to be as the 3 rhamnoside. Other compounds isolated by Blanc et al (1972) include leucocyanidol, quercitol, camphol, anda quercitol derivative containing a rhamnose moiety.

Gupta and Garg (1966) showed that myricyl alcohol was present in the leaves and stems of the plant. They also reported the presence of ellagic acid along with triancontane and hentriacontane (Power et al, 1913; Gupta et al, 1966). Watt et al (1962) reported the presence of hydrocyanic acid in both the vegetative and reproductive organs of the plant, though the plant has not yielded positive cyanogenetic tests. Ridet and Chatol (1964)

reported the presence of 0.23% of tannin in the fresh plant as well as resins (possibly euphorboresin and euphorbon or an isomer of either).

The following compounds obtained from the plant have been characterized: cycloartenol, 24-methylene-cycloartenol, β-sitosterol, euphorbol hexacozonate, β-amyrin acetate, 1-hexacosanol, ingeno-triacetate, tinyatoxin, 12-deoxy-4β-hydroxyphorbol-13-dodecanoate-20-acetateand 12-deoxy-4β-hydroxyphorbol-13-phenylacetate-20-acetate (Baslas et al, 1980;1981).

1.4.1 OBJECTIVE OF THE STUDY

The herb, E.hirta Linn has had wide and varying folkloric uses in the tropics and particularly in West Africa. All parts of the plant have been used either singly or in combination to cure ailments ranging from diarrhoea and dysentery, helmintic infestation, gonorrhoea, fever, aid lactation and even as an aphrodisiac to increase libido. However, inspite of this rather diverse activity, very little pharmacological evaluations have been done to ascertain the rationale behind most of the folkloric claims of its efficacy. The phytochemical work done so far has not been able to ascertain the principles responsible for some of the actions of this plant.

It is with this view in mind, that this study was designed to evaluate some of the folkloricclaims of the traditional practitioners concerning the use of the leaves of the plant; and if possible to isolate and purify the active principle(s) which is/are responsible for some of these actions. The pharmacological activity of the leaves of the plant will be studied using in vitro methods ie. tissue preparations or in vivo methods ie. whole animal studies. These studies will be designed in line with the folkloric claims of the plant. Other pharmacological properties (and mechanism of action) of the leaves of E.hirta will be investigated.

1.4.2 SCOPE OF PHARMACOLOGICAL SCREENING

The extraction of the leaves will be carried out using petroleum ether, ethanol and deionized water as extracting solvents. Fractions from the ethanol extract will be isolated (in collaboration with Dept. of Med. Chemistry) and also tested for phamacological activities. The crude extracts will be tested on the following assay systems.

1 IN VITRO STUDIES

A Isolated tissue studies

i) Effect of the crude extracts and fractions on the smooth muscles of the rabbit jejunum.

ii) Effect of the crude extracts on the smooth muscles of the guinea pig atrium.

B Haematological studies

i) Effect of the crude extracts on platelets in platelet rich plasma.

ii) Effect of the crude extracts on platelet adhesion in platelet rich plasma.

2. IN VIVO STUDIES

Whole animal studies

i) Effect of crude extracts on the blood pressure of anaesthesized cat.

ii) Effect of extracts on urine output and urinary electrolytes in rats.

iii) Determination of the lethal dose, LD_{50} in mice.

It is hoped that from the results of these screenings and the phytochemical tests:

a) Some scientific rationale would be established to support the treatment of some of these ailments, for which E.hirta is claimed to be of use in traditional medicine.

b) Other pharmacological properties of the plant will also be examined.

CHAPTER TWO

MATERIALS AND METHODS

2.1 PLANT MATERIAL

2.1.1 Collection and Identification

The leaves of Euphorbia hirta were obtained from Ahmadu Bello University (Main Campus) in Zaria, Kaduna State. They were collected and identified by the Hebarium keeper. Mr. M. Musa of the Department of Biological Sciences, Faculty of Sciences, A.B.U. A voucher specimen of the plant was deposited with the Herbarium under voucher number (Ohaeri Coll No. 1360).

2.1.2 Preparation

A large quantity of the fresh leaves of the plant were obtained and left to dry at room temperature for about a week. They were then weighed, several times until the weight was constant. The dried leaves were then ground into a fine powder with a wooden mortar and pestle and the powder kept in an airtight glass container, ready for use in the extraction procedure.

2.2 EXTRACTION PROCEDURE

35g of the dried and powdered leaves of E.hirta were defatted with petroleum ether and then extracted with ethanol and deionized water. The powder was soaked in petroleum ether (200ml) and allowed to stand at room temperature for 24 h. After thorough mixing, the mixture was filtered with Whatman paper No. 1 and the residue further extracted with ethanol (200 ml). The residue from ethanol extraction was then finally extracted with deionized water.

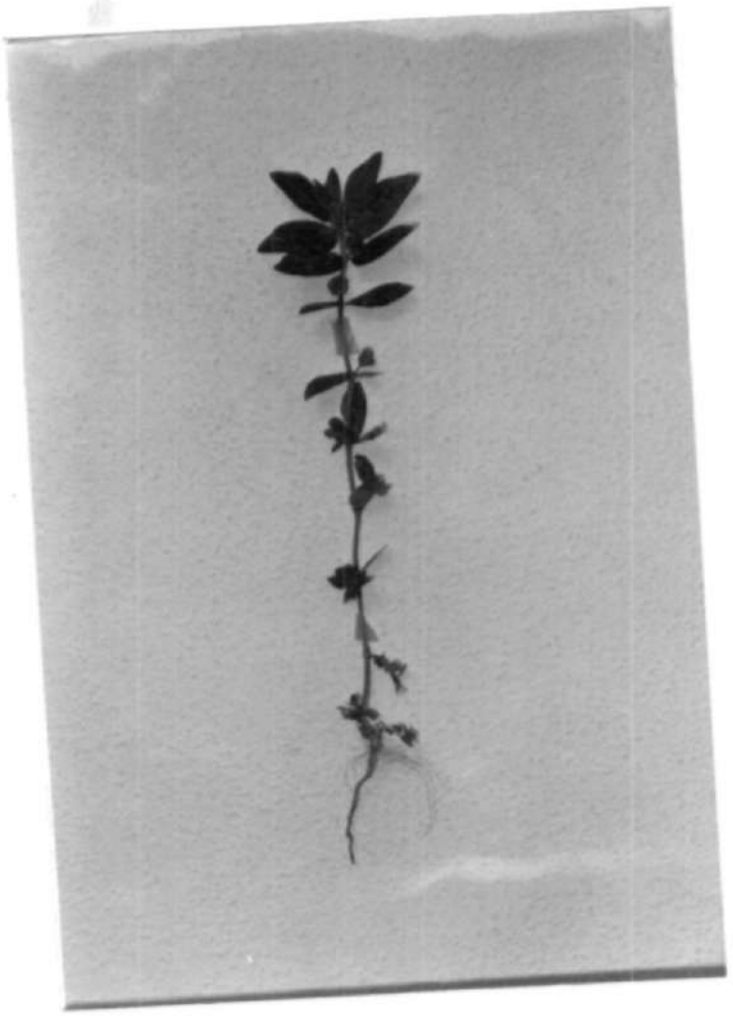


Plate I: A Euphorbia hirta plant

The extracts from the three solvents were dried and respectively labelled as petroleum ether, ethanol and water extracts. The petroleum ether extract was not used in this study because of the insolubility in either water or normal saline (0.9% w/v Nacl). This was justified because the local remedy containing E.hirta is prepared in either water or local alcohol, but not in petroleum ether.

2.3 ISOLATION

The column (100 x 3.5 cm) was packed with silica gel (60 - 120 u) and equilibrated with hexane for 24 h. The dried ethanol extract (3g) was loaded onto the column and eluted with 250 ml hexane: ethylacetate (20:1); 250 ml hexane: ethylacetate (10:1); 250 ml hexane:ethylacetate (1:5); 500 ml hexane: ethyl acetate (1:1) and finally collected over a period of 24 h. This was supposed to be a collaborative project with the Dept. of Med. Chemistry, A.B.U., Zaria Solvent(s) in each fraction were evaporated, dried and tested for biological activity.

2.4 DRUG SOLUTIONS AND CHEMICALS USED

2.4.1 Preparation of extract solutions

Stock solutions of the extracts were prepared by taking a weighed amount of the dried extract and dissolving in a measured volume of normal saline (0.9% w/v Nacl) solution.

2.4.2 Reference drugs used

The following standard drugs used were obtained from the various sources indicated below. Those in the solid state were prepared by dissolving weighed amounts in a measured volume of normal saline (0.9% w/v Nacl) solution.

Acetazolamide	Lederle Laborites Division
Acetylcholine chloride	Sigma Chemical Company
Adrenaline	Lifepharm Ltd.
Aprotinin	Sigma Chemical Company
Arachidonic acid	Sigma Chemical Company
Atropine sulphate	Halewood Chemical Ltd.
Stilbosterol dipropionate	May & Baker Ltd.
Ethanol (absolute)	ECCL Chemicals Ltd.
Frusemide	Lifepharm Ltd.
Indomethacin	Prof T. Y. Shen. University of Virginia, U.S.A.
Methanol	ECCL Chemicals Ltd
Methoctramine	Dr. I.M. Hussaini, A.B.U. Zaria, Nigeria.
Naloxone	Dr. Steven Hart, King's College University of London
NDGA	Sigma Chemical Company
Platelet Activitating Factor (PAF)	Bachem, Torrance. USA
Pentobarbitone sodium	May & Baker, Ltd.(Sargatal$^{(R)}$)
Petroleum ether	ECCL Chemicals Ltd.
Pirenzepine	Sigma Chemical Company
Propranolol	Sigma Chemical Company
Sodium chloride	BDH Ltd.
WEB 2086	Prof T. Y. Shen. University of Virginia U.S.A.

2.4.3 Storage of Drug solutions

Fresh solutions of the reference drugs were prepared for each day's work where this was necessary. Photosensitive drugs like adrenaline were kept in dark, amber sample bottles. Prepared stock solutions of the extract were tightly covered and refrigerated.

2.4.4 PHYSIOLOGICAL SOLUTIONS

1. Kreb-Ringer solution was used for the innervated guinea-pig atrium (Kitchen, 1984 Experimental Pharmacology, 1991).
2. Tyrode solution was used for the rabbit jejunum (Kitchen, 1984; Experimental Pharmacology, 1991).
3. Normal saline served as the control solvent medium for all the drugs and extracts used in the experiment.
4. McEwen's solution was used for the guinea pig atrium (Kitchen, 1984).

(See Appendix I for the composition of physiological solutions used)

2.5 PHARMACOLOGICAL SCREENING

Test Animals

Adult male and female albino rats and mice (Wistar strain) were brought from the Vom Veterinary Institute, Plateau state for the studies. These were kept in the Animal House of the Department of Pharmacology and Clinical Pharmacy of Ahmadu Bello University, Zaria. They were fed on mouse pellets - which is a concentrate with a high protein content. Water was supplied to the animals in standard drinking bottles.

Adult male rabbits and guinea pigs were obtained locally from the market and kept in the Animal House of the same department for a week prior to use. They were fed on green vegetables. Adult male cats similarly obtained locally were fed on milk and fish.

2,6 INSTRUMENTATION

2.6.1 Auxiliary Apparatus

1. Syringes and needles were used for parenteral administration of appropriate drug solution(s) to test animals.
2. Butterfly intravenous needles and accessories were used for withdrawal of blood from donor.
3. Plastic Metabolic cages (Griffin and George) capable of separating urine from faecal matter were used in the diuretic studies carried out on the rats.
4. Rubber cannulae were used to cannulate the carotid artery and femoral veins of the cat.
5. Churchill Thermoregulator (serial No. S/00052/27) was used for controlling temperature in the organ bath.
6. Centrifuge (model ECONOSPIN 1200010) was used for centrifuging blood samples.

2.6.2 Recording Apparatus

1. The recording microdynamometer (Ugo Basile, model No. 7050) was used to record the isometric contractions and relaxations of the various tissue preparations.
2. The flame photometer (model Cornning 400) available at the Chemical Pathology Department of Ahmadu Bello University Teaching Hospital, Zaria was used for measuring electrolyte concentrations.

3. The pH meter (Pye Unicam model 292) in the above department was used for measuring pH of urine samples.

4. The Chronolog electronic impedance model 530 VS (Coulter Electronic Ltd) coupled to an Omniscribe series (D5000) recorder was used for measuring platelet aggregation.

5. The Electrophysiograph (PCM Narco Biosystems) for blood pressure measurement.

2.7 METHODOLOGY

IN VITRO TESTS

2.7.1 Isolated Tissue Preparations

The isolated tissues used in all the experiments were carefully removed from freshly killed animals. These were quickly placed in petri dishes containing well aerated, physiological solutions. Aeration varied with the type of tissue, it could either be air or oxygen or carbogen (95% 0_2 & 5% CO_2 mixture). The physiological solutions also varied with the type of tissue used. These tissues were then set up in organ baths (20 ml volume) and allowed to stabilize for about 30 to 60 minutes before the administration of drugs for screening purposes. The microdyanometer (model no. 7050) was connected to appropriate transducers and used in recording responses on Ugo Basile paper (Kitchen, 1984).

The Churchill Thermoregulator kept the temperature of the set-up constant at 37°C. Standard drugs and extracts were injected into the organ bath with syringes and needles and their pharmacological effects studied. After the administration of each drug or extract, the tissues were washed three times and allowed to rest for 5 minutes before the addition of another drug or extract.

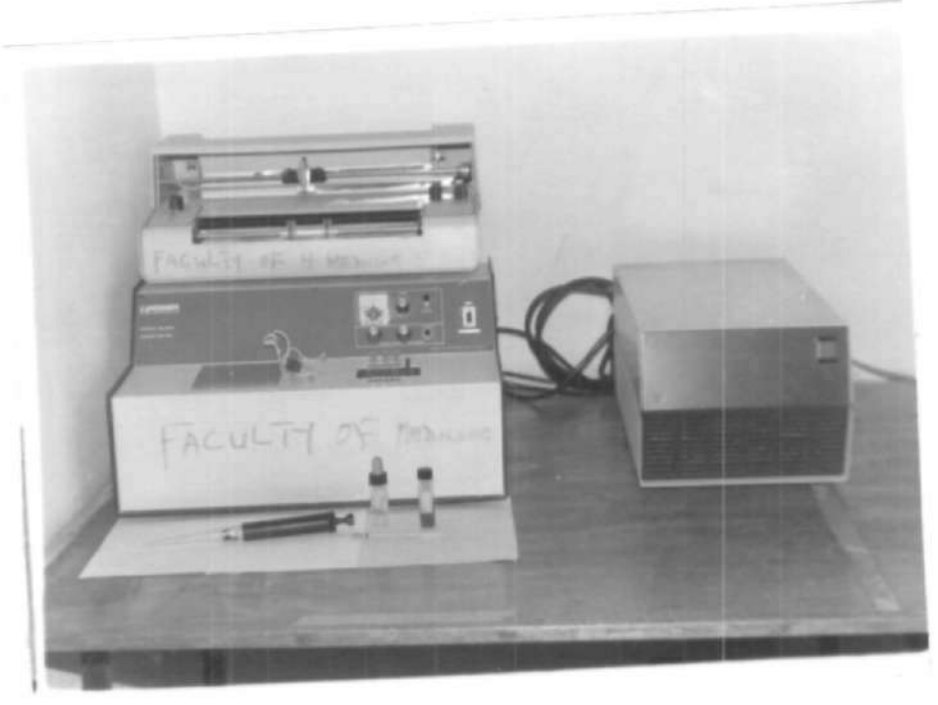


Plate II: The Chronolog Electronic Impedance Aggregometer

2.7.1A Rabbit Jejunum - Finklemann preparation

This preparation provides a method for the investigation of the effect of drugs on the parasympathetic and sympathetic nervous system. The rabbit jejunum exhibits spontaneous activity. This strong and consistent response of the intestine is used for the study of relaxation produced by sympathomimetic drugs.

Method

Male rabbits weighing between 3 - 5 kg were killed by dislocating the neck and exsanguination. The abdomen was quickly opened and the gut (or jejunum) removed. The jejunum was placed in a large petri dish containing well aerated Tyrode solution. The attached mesentery and fat were trimmed off. A suitable segment of the jejunum (about 1 - 1½ in) was removed and mounted in an organ bath of 20ml volume. The fluid was aerated with 95% 0_2 & 5% $C0_2$ mixture. The weight and magnification of the lever was adjusted, so as to produce uniform spontaneous contractions of the gut of approximately 3 - 4 cm in amplitude.

The effects of the water (0.025 - 2.5 mg/ml) and ethanol (0.25 - 2.5 mg/ml) extracts and fraction 28 (from ethanol extract fractionation) (0.1 - 0.5 mg/ml) were tested in turn. Adrenaline (2.5 - 250 mg/ml) was used as a reference. The effect of some antagonists namely pirenzepine (5 μM), atropine (1μM), propranolol (1μM) on the extracts and fraction 28 were also tested. These were injected into the reservoir and allowed to come in contact with the tissue for 5 minutes before extract injection. Washing is accomplished by overflow as exposure of the tissue to air disrupts spontaneous activity (Kitchen, 1984; Experimental Pharmacology, 1991).

2.7.1B Guinea pig atrium

Male guinea pigs (300 - 500g) were killed by cervical dislocation and exsanguination. The heart was removed as rapidly as possible and plunged into ice-cold McEwen's solution. About 1 cm of aorta was removed intact. The heart was immersed in Ringer-Locke's solution and squeezed firmly to expel blood from the organ and prevent the formation of clots. Excessive connective and lung tissues were carefully trimmed off; and the heart was re-immersed in ice-cold Ringer solution and the ventricles squeezed again. The heart was then transferred to a petri-dish containing ice-cold aerated Ringer's solution. The pericardium and fat were removed and the ventricles were carefully dissected off so as not to damage the pacemaker. An atrium was then mounted vertically in an organ bath of 20 ml volume. The fluid was aerated with 95% O_2 and 5% CO_2 mixture. (Kitchen, 1984; Experimental Pharmacology, 1991).

The contractions of the atrium was left to stabilize for about 30 minutes. The effect of the water extract of E.hirta (0.25 - 2.5 mg/ml) was tested. The effects of some standard references namely acetylcholine (0.025 - 0.5 μg/ml) and choline (0.025 - 0.25 mg/ml) were tested. The effects of these drugs and extracts in the presence of the antagonist, methoctramine (0.6 μM) were also investigated. The antagonist was injected into the reservoir and allowed to come in contact with the tissue for 5 minutes before administration of the extract or drugs.

2.7.2 HAEMATOLOGICAL STUDIES

2.7.2A Collection of blood

Blood was collected by clean venapuncture (antecubital vein) of human volunteers who had not ingested aspirin-like drugs during the previous fortnight. Blood (18 ml) was

withdrawn into plastic syringe and further divided into 2 plastic tubes, containing 1 ml of trisodium citrate 3.8% w/v (1:9 w/v).

2.7.2B Effect of leaf extracts of E hirta on platelets in platelets rich plasma (PRP)

Preparation of PRP from blood

The blood obtained above was then gently mixed with trisodium citrate; this was centrifuged at 250g for 10 minutes in a centrifuge model ECONOSPIN 1200010 at room temperature to obtain the platelet rich plasma (PRP). PRP was separated and stored at room temperature in polypopylene vials before use. The time interval between blood collection and assay of platelet aggregation in PRP was less than 30 min.

Measurement of Platelet adhesion in PRP

Platelet aggregation was carried out on samples of PRP (1.0 ml) contained in siliconized glass tubes using a Chronolog electronic impedance aggregometer which measures platelet adhesion to the platinum wire electrode. PRP was maintained at a temperature of 37°C and stirred by means of a teflon magnetic stirbar rotating at 1000 revolutions per min (rpm) (Hussaini, 1986).

Platelet adhesion or aggregation was displayed on an Omniscribe series D5000 recorder. Aggregation was induced with standard drugs: namely Platelet activating factor (PAF 50nM - 1μM) and adrenaline (1 - 10μg/m). The effect of water (50μg - 1 mg/ml) and ethanol (50μg - 5mg/ml) extracts were studied in the absence and presence of the above aggregating agents. Subthreshold concentrations of the extracts were added 2 minutes prior to the addition of the aggregating agent to check for any antagonistic effect. The aggregation produced by the extract was tested in the presence of the anti-aggregating agent,

Indomethacin (0.014 - 0.14μM). The effect of WEB 2086 (10.87 - 21.74 nM) - a known, selective PAF antagonist on PAF and extract-induced platelet aggregation was studied. The effect of nifedipine (0.23 μM) on the extract-induced platelet aggregation was also studied. Experiments were always started 30 mins, after blood collection and completed within 3h.

IN VIVO TESTS

2.7.3 WHOLE ANIMAL STUDIES

2.7.3A The effect of the crude extracts on the blood pressure of an anaesthesized cat

Male cats weighing (1.8 - 2.5 kg) were used in this study. The cats were weighed and anaesthetized with pentobarbitone sodium (40 mg/kg) given intraperitoneally. The carotid artery and femoral vein were cannulated. Heparin (10U/ml) was injected through the femoral vein before cannulation of the carotid artery for blood pressure recording. The carotid artery was connected to the physiograph via the cannula for blood pressure measurement. Recordings were made on a narcobiosystem paper. Control blood pressure was recorded on the physiograph.

The effect of normal saline (2 ml) and standard reference drugs namely acetylcholine (0.005 - 0.5 μg.kg) choline (0.5 - 1mg/kg) and isoprenaline (0.5 - 2μg/kg) were tested. The effects of the water (0.05 - 5mg/kg) and ethanol (0.5 - 10 mg/kg) extracts were studied. Drugs were injected through the femoral vein. The effect of these drugs and extracts in the presence of antagonists was also studied. The antagonists used were atropine (5 &10 μg/kg) and pirenzepine (5mg/kg). After the addition of any drug, the blood pressure was allowed to stabilize before the next addition(s). All drugs were injected in 0.5ml normal saline, and not more than 2ml was injected at a time. Heparinized saline was injected through the cannula to prevent blood clotting.

2.7.3B Diuretic effect of extracts on Urine Output and Electrolytes

Adult albino rats (Wistar strain) were used for diuretic studies on the water extract of the leaves of E.hirta (administered intraperitoneally). Each set consists of four albino rats. The rats were separated into metabolic cages capable of collecting uncontaminated urine void of faecal matter. They were supplied with drinking water and food ad libitum. Normal saline (0.25ml) was administered to each one intraperitoneally. The urine output was measured at 30 mins, 1h, 3h, 6h, 12h and 24h. These constitute the basal readings.

After 24h, four of the rats were injected with frusemide (5mg/kg), another set was given acetazolamide (5 mg/kg); ethanol extract (50mg/kg and 100 mg/kg) was given to the third and fourth sets respectively; the fifth and sixth sets received water extract (50 mg/kg and 100 mg/kg) respectively, whereas normal saline was given to the last set. The urine output was measured at the same time intervals as stated for the basal reading.

In all cases, urine excreted up to 24h for each rat was analysed and the Na^+, K^+, HCO_3^- and Cl^- contents were determined using a flame photometer (Cornning 400 model). The pH was also determined using PYE UNICAM 292. The results were tabulated and graphically illustrated as cumulativeurine output versus excretion time. Estimation of diuretic activity was based on a modification of the method described by Kau et al, 1984.

2.7.3C Acute Toxicity Test

(Determination of lethal dose, LD_{50})

A pilot study was conducted to determine the maximum dose of each extract that did not produce death (0.2g/kg for water extract and 0.5g/kg for ethanol extract) and minimum dose that caused 100% death (5.0g/kg) and other doses were selected within these ranges. The extracts were injected intraperitoneally.

Male mice (18 - 28g) were fasted overnight but allowed water. Six groups of five mice were randomly selected and placed in different cages for each leaf extract. Each group

of five animals received a dose of the extract within the dose range, and one group (control) received normal saline (0.25 ml). Signs and symptoms of toxicity in the mice were observed and recorded. The onset of each symptom and time of death within a 24h period were also noted. The doses that killed 50% of the population (LD_{50}) were calculated by the arithmetic methods of Karber, as modified by Aliu and Nwude (1982), and that of Reed and Muench (1938) as well as the graphical method of Litchfield and Wilcoxon (1949).

The mechanism by which E.hirta leaf extracts induced death was investigated. Three sets of 5 mice were fasted overnight. They were pretreated the following day with atropine (10, 50 and 100μg/kg) respectively for 1h before the administration of the dose of water extract that produced 100% mortality. The mice were observed for a 24h period. Pretreatment with other antagonists were also studied, namely naloxone (0.5mgkg); indomethacin (0.5, 1mg/kg); hydrocortisone (100, 200 μg/kg); WEB 2086 (0.5, 1.0mg/kg); aprotinin (10,000; 20;000 U/kg) and NDGA (0.5; 2 mg/kg).

2.7.4 **Statistical Method**

Results show means ± S.E.M. (with the number of observations shown in parenthesis) where:

$\bar{x}$	=	mean of the sample (x)
n	=	number of observations
d	=	deviation from the mean
S.D	=	Standard Deviation

$$S.D_{(n-1)} = \sqrt{\frac{(x-\bar{x})^2}{n-1}}$$

$$S.E.M = \frac{SD_{(n-1)}}{\sqrt{n-1}}$$

CHAPTER THREE

RESULTS

3.1 PLANT MATERIAL PREPARATION

The quantity of the dried, powdered leaves of Euphorbia hirta are shown in Table 3.2. The powdered leaves are green in colour.

3.2 EXTRACTION

The quantity of powdered leaves of E.hirta and % yield are represented in Table 3.2. The water extract had a musty odour and the ethanol extract had a sweet smelling flavour.

3.3 PHARMACOLOGICAL STUDIES

3.3.1A The effect(s) of water and ethanol extracts of the leaves of E. hirta on rabbit jejunum

The representative tracings are shown in Fig. 3.3.1.1.Both water (0.025 - 2.5 mg/ml) and ethanol (0.25 - 1mg/ml) extracts produced dose-dependent relaxations. The maximum relaxation attained with water extract was 95.8% at the concentration of 2.5 mg/ml with a resultant average of X_{H_2O} = 94.5 ± 0.9% (n = 3) [Table 3.3.1.]. The corresponding value for the ethanol extract was 92.3% at 2.5 mg/ml with a resultant average of 87.6 ± 7.9% for X_{EtOH}(n = 3) [Table 3.3.1.2]. The maximum relaxationobtained for fraction 28 (one of the fractions of the ethanol extract) was 80.4% at 0.5mg/ml with a resultant average of 70.4 ± 4.2% for X_{28} (n = 3) [Table 3.3.1.3]. The EC_{50} values for adrenaline, fraction 28, ethanol and water extracts are 45 ± 2.41ng/ml,0.2 ± 0.01 mg/ml, 0.3 ± 0.04 mg/ml and 0.08 ± 0.01 mg/ml (n = 3) respectively.

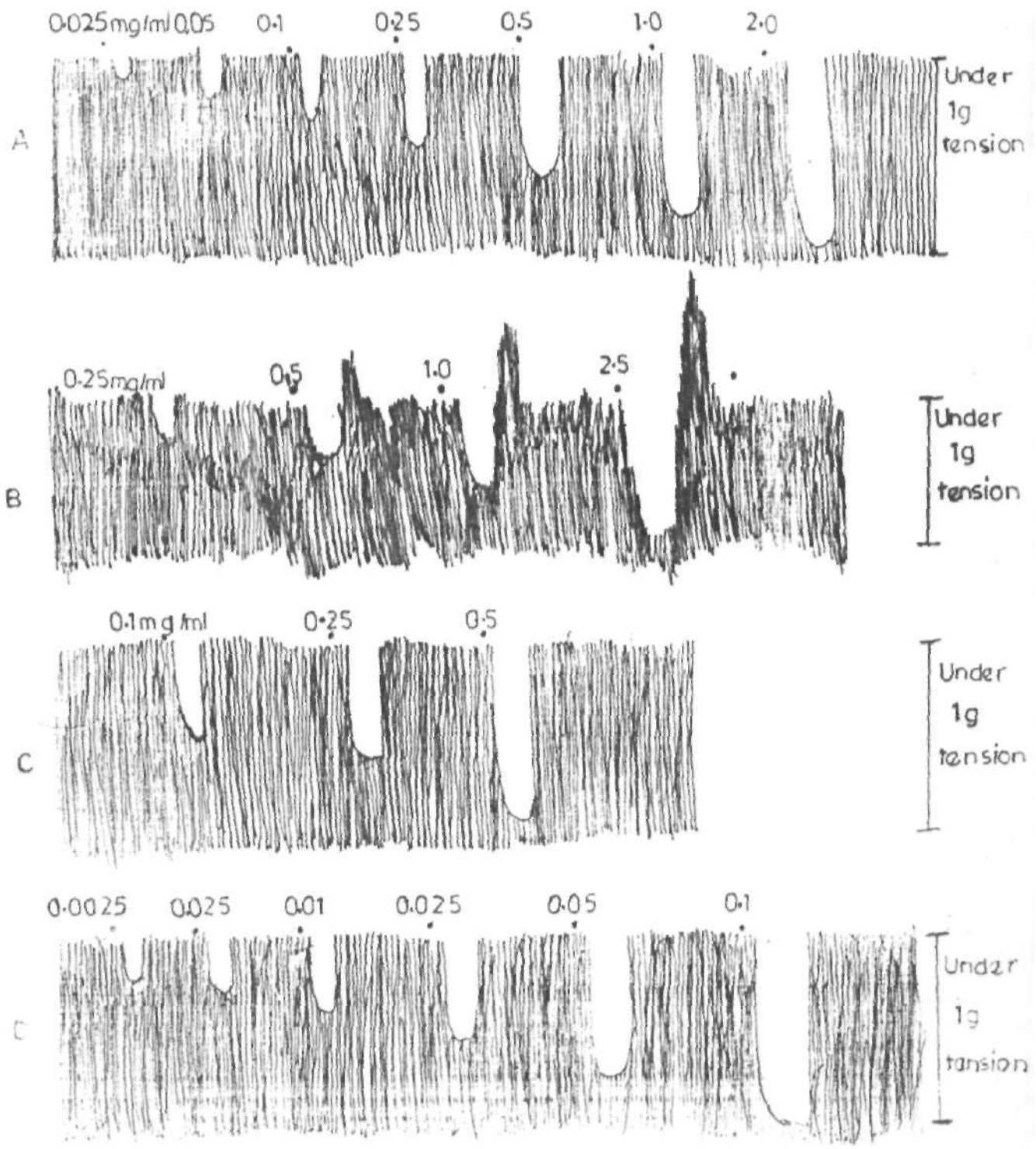


FIG. 3.3.11 Tracings showing the dose-related effects of H_2O (A) and Ethanol (B) extracts of E. hirta leaves, fraction 28 (C) and adrenaline (D) on the spontaneous activity of rabbit isolated jejunum.

Table 3.2: Physical properties of the water and ethanol extracts of E.hirta

	Water extracts	Ethanol extracts
Quantity of powder taking (g)	35.0	34.0
Volument of solvent (ml)	200	200
Yield (q)	2.3	1.5
Yield (%)	6.5	4.41
Colour	Dark green	Brown
Colour in normal saline solution	Dark green	Brown

Table 3.3.1.1: Average % relaxations obtained with water extract of the leaves of E.hirta on the rabbit jejunum

Concentration of H_20 extract (mg/ml)	Average % relaxation (X_{H_20}) $\pm$ S.E.M
0.025	33.2 $\pm$ 0.8
0.050	44.0 $\pm$ 0.0
0.100	62.9 $\pm$ 0.0
0.250	76.6 $\pm$ 12.4
0.500	89.3 $\pm$ 2.9
1.000	89.8 $\pm$ 1.1
2.500	94.5 $\pm$ 0.9

Two of the numerous experiments on the water extracts showed slight contractions; but this was disregarded as responses to other drugs including the standard reference drugs - adrenaline were not significant. The relaxations produced by the water extract were prominent even at very low doses (as low as 0.025mg/ml), % relaxation was 33.2%. This increased steadily to 76.6% at 0.25mg/ml. The increase between 0.5 - 2.5mg/ml was however not appreciable (89.3 - 94.5%) [Table 3.3.1.1].

The ethanol extract showed biphasic action; though the relaxations were more prominent than the contractions. In comparison with the water extract, the relaxations were less than those obtained at the same dose with water. The relaxations (which consistently preceeded the contraction of each dose) are shown in Table 3.3.1.2a. The contractions obtained with ethanol are shown in Table 3.3.1.2b.

Fraction 28 is one of the fractions obtained from the fractionation of the ethanol extract. It is the 28th fraction eluted from the column, and it produced only relaxation on this isolated tissue (Table 3.3.1.3). Other fractions from the column chromatography with the exception of fractions 25 to 27 (which produced slight relaxations); induced contraction or no response on the tissue.

Table 3.3.1.2a: Average % relaxations obtained with ethanol extract of the leaves of E.hirta on the rabbit jejunum

Concentration of EtOH extract (mg/ml)	Average % relaxation (x_{EtOH}) ± S.E.M
0.25	46.8 ± 6.87
0.50	65.6 ± 3.11
1.00	79.4 ± 2.31
2.50	87.6 ± 1.91

Table 3.3.1.2b: Average % contractions obtained with ethanol extract of the leaves of E.hirta on the rabbit jejunum

Concentration of EtOH extract (mg/ml)	Average % contraction (x_{EtOH}) ± S.E.M
0.25	34.2 ± 0.64
0.50	44.3 ± 0.40
1.00	67.5 ± 6.0
2.50	100 ± 0.0

Table 3.3.1.3: Average % relaxations obtained with fraction 28 from the ethanol leaf extract of E.hirta on the rabbit jejunum

Concentration of fration 28 (mg/ml)	Average % relaxation (x_{28}) ± S.E.M
0.10	40.9 ± 8.43
0.25	61.0 ± 3.46
0.50	70.4 ± 4.20

Table 3.3.1.4: Average % relaxations at different doses with adrenaline on the rabbit jejunum

Concentration of adrenaline (μg/ml)	Average % relaxation (x_{Adr}) ± S.E.M
0.0025	17.1 ± 2.77
0.0050	21.4 ± 7.28
0.0100	18.9 ± 2.93
0.0250	20.0 ± 2.25
0.0500	74.7 ± 9.99
0.1000	87.8 ± 3.87
0.2500	80.6 ± 0.01

Adrenaline, a sympathomimetic drug was the standard drug used and this produced dose-dependent relaxations as shown in Table 3.3.1.4.

The % maximal and minimal response of the extracts and the standard adrenaline were compared as shown in Fig. 3.3.1.2a & b. At low concentration, the water extract (0.025mg/ml) is nearly as potent as the ethanol extract (0.25mg/ml) which is ten times its concentration. Fraction 28 (0.1mg/ml) produced 40.9% relaxation though it is only a third of the concentration of ethanol extract used. The concentration-dependent relaxation obtained with the standard sympathomimetic-adrenaline gave a maximum of 87.8% at a concentration of 0.1μg/ml - so it is about a 1000 x more potent than the extract. It is also shown that at the high concentration (2.5mg/ml), the water and ethanol extract had different % maximal relaxation (94.5% and 87.6% respectively) even though the concentrations are the same (2.5mg/ml). The water extract was more potent than the ethanol extract, this maybe due to the presence of the spasmodic principle in the latter, which causes contraction. However, fraction 28 - a fraction from the ethanol extract produced only relaxations within the concentration range used.

3.3.1b **Mechanism of E.hirta leaf extract induced relaxations on rabbit jejunum**

In these studies, three antagonists were used, atropine (a nonselective muscarinic receptor antagonist), pirenzepine (a selective muscarinic receptor antagonist and a non-selective β- adrenoceptor antagonist - propranolol. The relaxations produced by the extracts and adrenaline were not affected significantly by atropine (10nM) but blocked by the nonselective β-adrenoceptor antagonist - propranolol (1μM). There was complete blockade at doses below 0.1mg/ml. However, an increase in the dose of water extract resulted in a decrease in percentage blockade from 87% (at 0.1mg/ml) to 47% (at 2.5mg/ml) [Table 3.3.1.5]. The EC_{50} was shifted from 0.08mg/ml to 4mg/ml ($n = 3$) in the presence of the β-adrenoceptor antagonist.

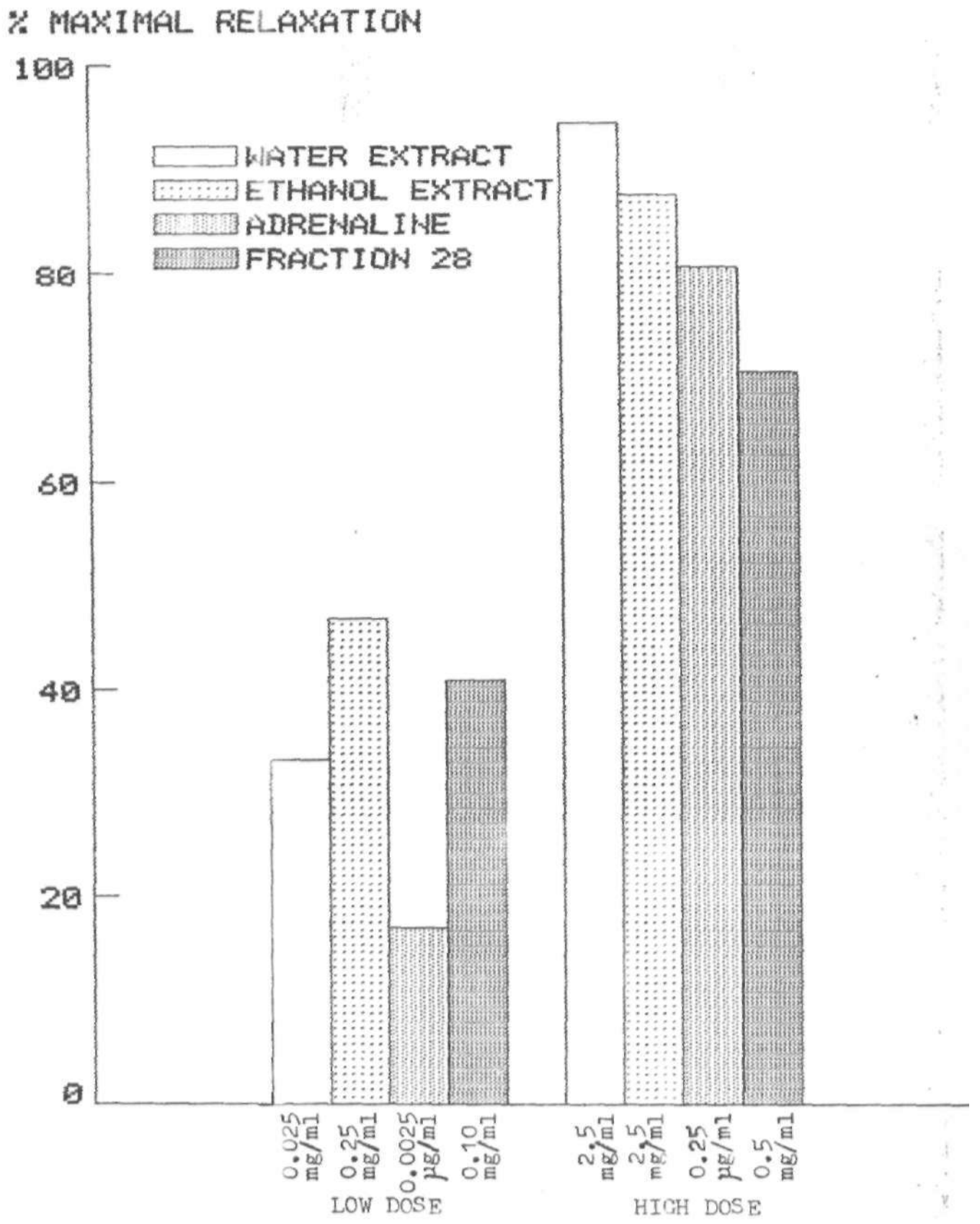


Fig. 3.312: Histogram representation of % relaxation produced at Low and High doses of water, ethanol extract of E. hirta, fraction 28 and Adrenaline.

Table 3.3.1.5: *% relaxation of rabbit jejunum produced by water extract in the presence and absence of propranolol (1μM)*

Dose (mg/ml)	% Max. response before Propranolol	% Max. response after Propranolol	% blockade
0.025	33.2 ± 0.8	0.0	100.0
0.050	44.0 ± 0.0	0.0	100.0
0.100	62.9 ± 0.0	8.15 ± 0.32	87.04
0.250	76.6 ± 0.1	11.25 ± 0.11	85.31
0.500	89.3 ± 2.9	21.85 ± 5.33	75.53
1.000	89.8 ± 1.1	36.90 ± 6.58	58.91
2.500	94.5 ± 0.9	49.30 ± 3.89	47.83

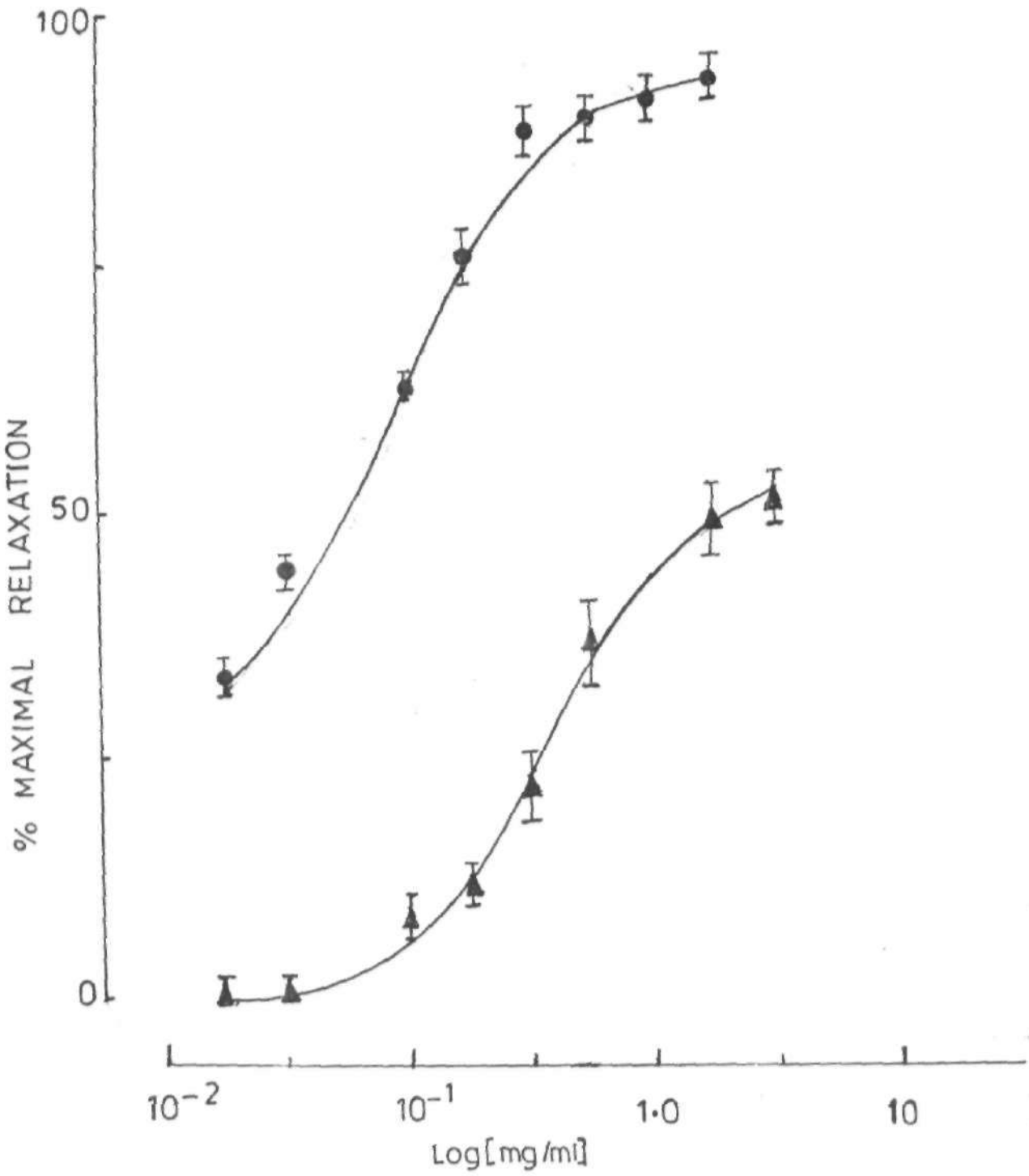


FIG. 3.3.1.3 Relaxation of spontaneous contractions of rabbit isolated jejunum produced by E. hirta leaf H_2O extract in the absence (●—●) and presence (▲—▲) of propranolol (1μM). Each vertical bar is ± s.e.m (n=3).

A graphical plot was made for the water extract before and after propranolol (Fig. 3.3.1.3). There was a shift of the curve to the right and a drop in the asymptote when propranolol (1μM) was added to the physiological solution.

3.3.1c **Antimicrobial Tests**

When the extracts and fraction 28 were incubated with E.coli and S. aureus, there was initial inhibition zones which disappeared after 24 h. Increasing the doses did not improve the antibacterial activity of the plant extracts.

3.3.2a **Effects of Platelet Activating Factor (PAF) and adrenaline on platelets**

PAF (50 - 100nM) and adrenaline (1-10μg/ml) caused a concentration - related aggregation of human platelets maintained in plasma. Typical aggregation responses are shown in Fig. 3.3.2.1a & b. The concentration of PAF and adrenaline required to produce 50% maximum aggregation using the impedance procedure in PRP are 80nM and 0.025M respectively.

3.3.2b **Effects of ethanol and water extracts of E.hirta leaves on platelets**

Ethanol extract (0.05 - 5mg/ml) had no aggregatory effect on human platelets. Water extract (10-100μg/ml) also did not produce platelet aggregation. However, higher concentrations(0.2 - 1mg/ml) of the water extract induced a concentration - dependent aggregation with EC_{50} value of 450 $\pm$ 2.49μg/ml (Table 3.3.2.1). Typical tracings are shown in Fig. 3.3.2.2. The platelet aggregation caused by the water extract was an irreversible, single wave.

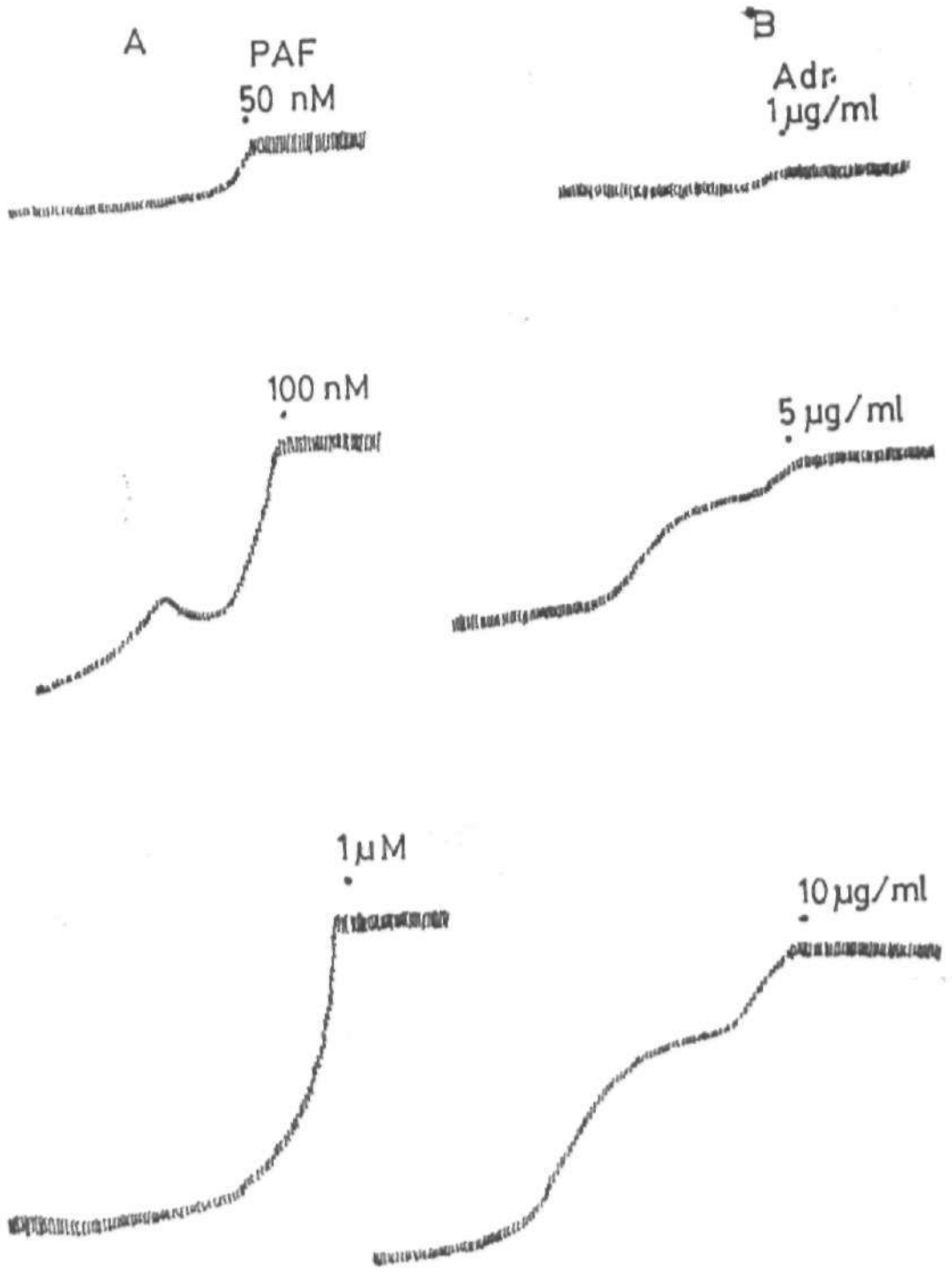


FIG. 3.3.2.1. : Effect of PAF(A) and Adrenaline(B) on platelets

3.3.2c **Effects of ethanol extracts and subthreshold concentration of water on PAF-induced platelet aggregation**

Maximum platelet aggregation was produced with PAF (1μM) and this was repeated in the presence of various concentrations (0.05 - 5mg/ml) of ethanol extract and subthreshold concentrations (50 - 200μg/ml) of water extract. The extracts were injected into the cuvette, 2 minutes before the addition of PAF (1μM). Both extracts had no observable effects on PAF-induced platelet aggregation (Table 3.3.2.2). In contrast, WEB 2086 - selective PAF antagonist, blocked the responses of the platelets to PAF (Table 3.3.2.3.)

Table 3.3.2.1: Effect of water (H_2O) extract on human platelets

Concentration of H_2O extract (μg/ml)	Mean of Platelet aggregation $\pm$ S.E.M
50.0	0.0
100.0	0.0
200.0	8.42 $\pm$ 1.49
300.0	15.79 $\pm$ 2.73
400.0	20.00 $\pm$ 1.01
500.0	100.0
750.0	100.0
1000.0	100.0

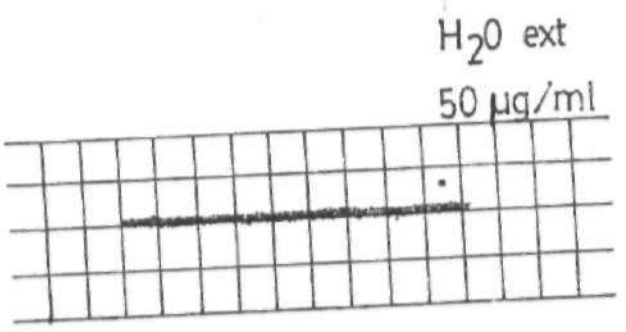
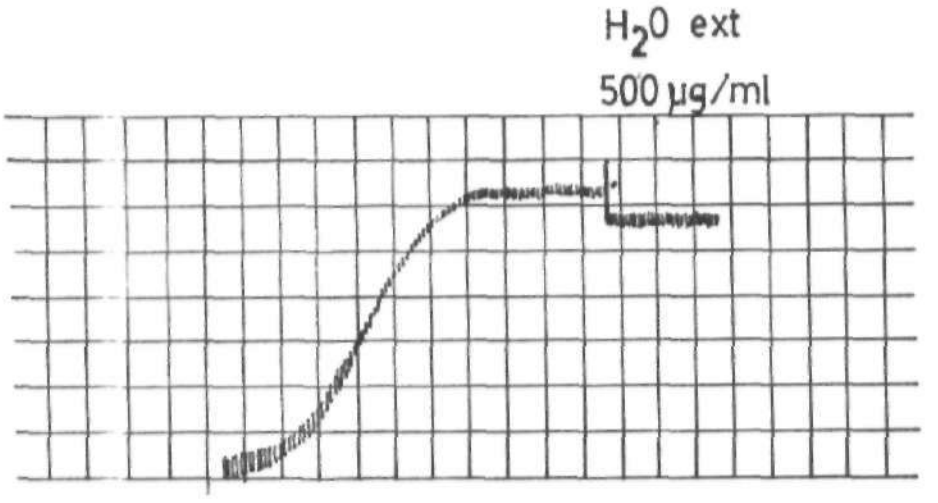
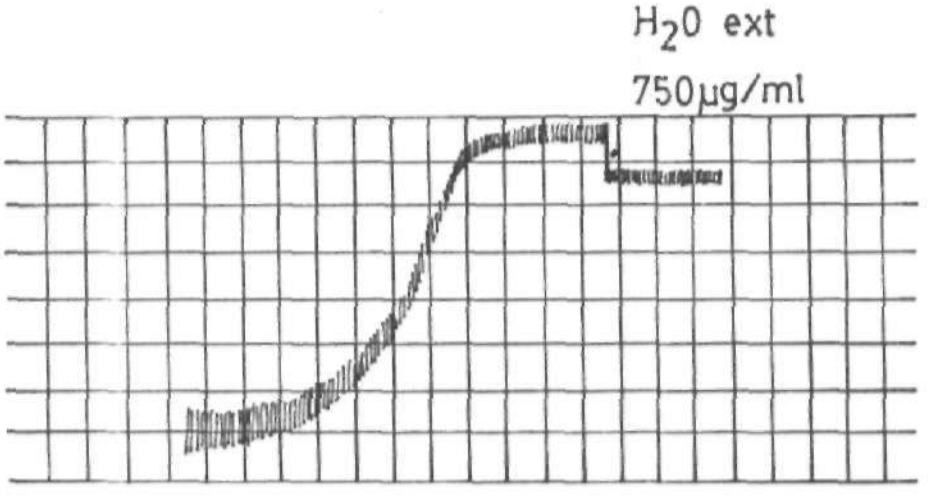


FIG. 3.3.2.2.a: Effect of water extracts of E. hirta on human platelets in PRP





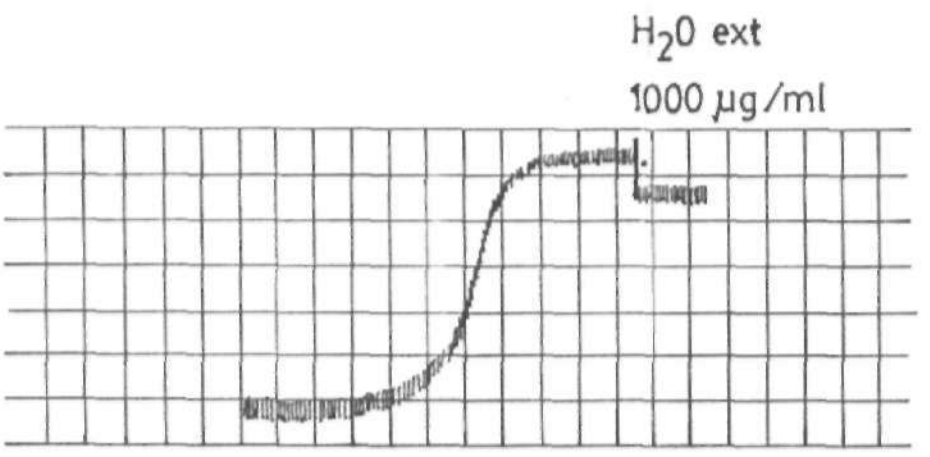


FIG. 3.3.2.2.b: Effect of water extracts of E. hirta on human platelets in PRP

Table 3.3.2.2: Effect of subthreshold concentration of water (H_2O) and ethanol (EtOH) extracts on PAF-induced aggregation

Concentration of PAF (μM)	Concentration of H_2O extract (μg/ml)	Mean % Platelet aggregation $\pm$ S.E.M
1.0	0.0	100
1.0	50.0	97.37 $\pm$ 1.41
1.0	100.0	100
1.0	200.0	97.61 $\pm$ 2.01

Concentration of PAF (μM)	Concentration of H_2O extract (μg/ml)	Mean % Platelet aggregation $\pm$ S.E.M
1.0	0.0	100.0
1.0	0.5	99.41 $\pm$ 0.06
1.0	1.0	98.67 $\pm$ 1.21
1.0	5.0	99.08 $\pm$ 0.17

Table 3.3.2.3: Effect of WEB 2086 on PAF-induced platelet aggregation

Concentration of PAF (μM)	Concentration of WEB 2086 (nM)	Mean % Platelet aggregation $\pm$ S.E.M
1.0	0.0	100
1.0	1.09	88.1 $\pm$ 1.63
1.0	10.90	0.0

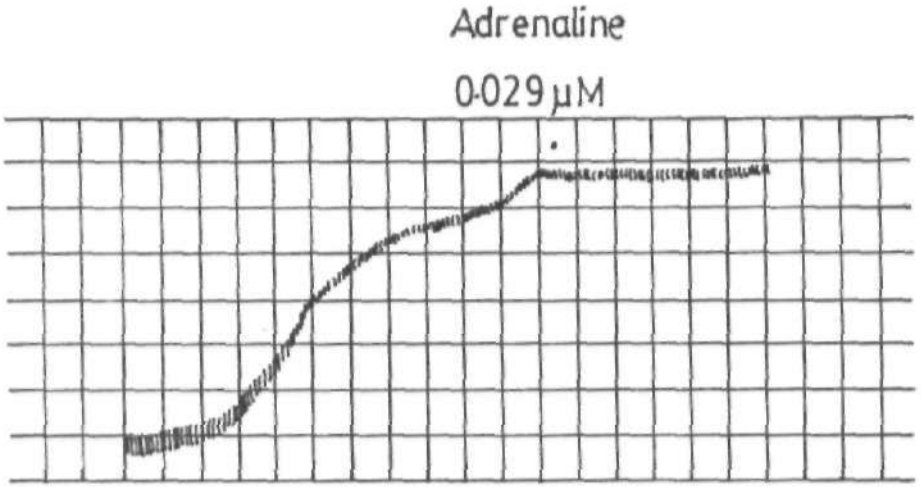
3.3.2d Effect of ethanol extract and subthreshold concentrations of water extract on adrenaline induced aggregation

Adrenaline (5μg/ml)induced a maximum platelet aggregation when added to PRP. Both water and ethanol extract had no significant effect on adrenaline-induced aggregation (Table 3.3.2.4). However, indomethacin (0.014μM) - a cyclooxygenase inhibitor-blocked the effect of adrenaline on platelets by 87.1% (Fig. 3.3.2.3).

3.3.2e Mechanism of water extract-induced platelet aggregation

Since at high concentrations (0.2 - 1mg/ml) the water extract produced platelet aggregation, the mechanism underlying this action was investigated. A concentration (500 μg/ml) of the water extract that produced maximum platelet aggregation was used in the presence of a number of standard inhibitors of aggregation in this study.

Indomethacin (0.014 - 0.14 μM) produced a concentration related inhibition of water extract-induced platelet aggregation (Fig 3.3.2.4). The EC_{50} value is 0.026 $\pm$ 0.005 μM. The aggregation could not be completely abolished even with 0.28 μM of the cyclooxygenase inhibitor. Nifedipine (0.23 μM) - a calcium channel blocker, also reduced the aggregation caused by the water extract by 13.7%. WEB 2086 (10.87 - 21.74 nM) had no significant effect on the water extract induced aggregation of human PRP.



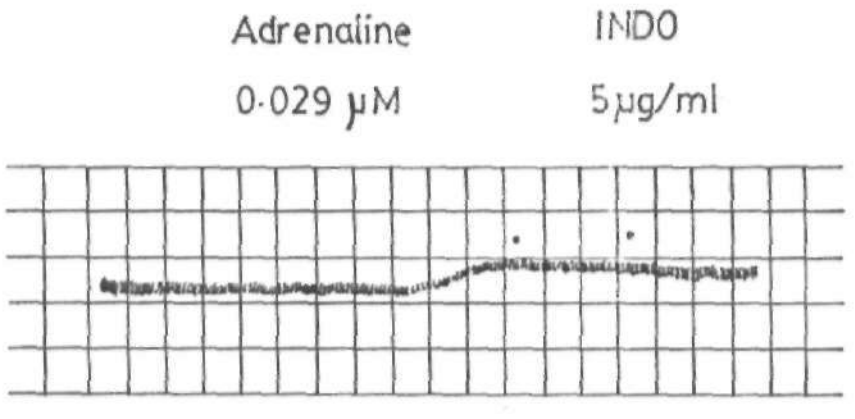
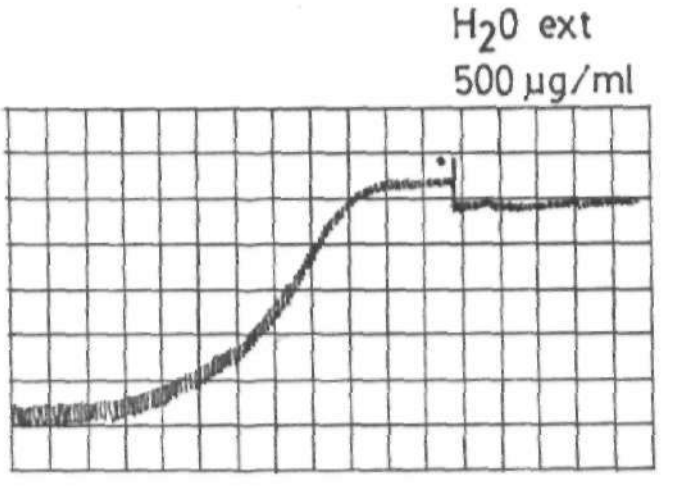


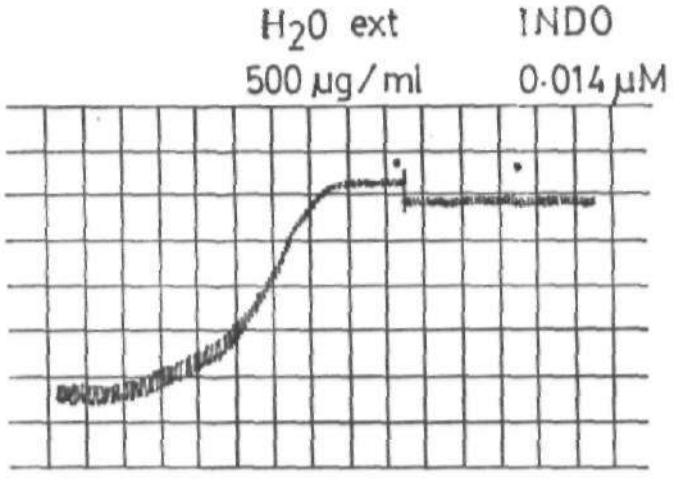
FIG. 3·3·23: Effect of Indomethacin (0·014 µM) on Adrenaline induced- platelet aggregation in PRP

Table 3.3.2.4: Effect of ethanol extract and subthreshold concentration of water extract on adrenaline-induced aggregation.

Concentration EtOH extract (mg/ml)	Concentration of Adr (μg/ml)	Mean % Platelet aggregation $\pm$ S.E.M
1.0	0.0	0.0
0.0	5.0	100.0
1.0	5.0	96.8 $\pm$ 1.31

Concentration H_2O extract (mg/ml)	Concentration of Adr (μg/ml)	Mean % Platelet aggregation $\pm$ S.E.M
0.2	0.0	8.42 $\pm$ 0.98
0.0	5.0	100.0
0.2	5.0	98.93 $\pm$ 0.98





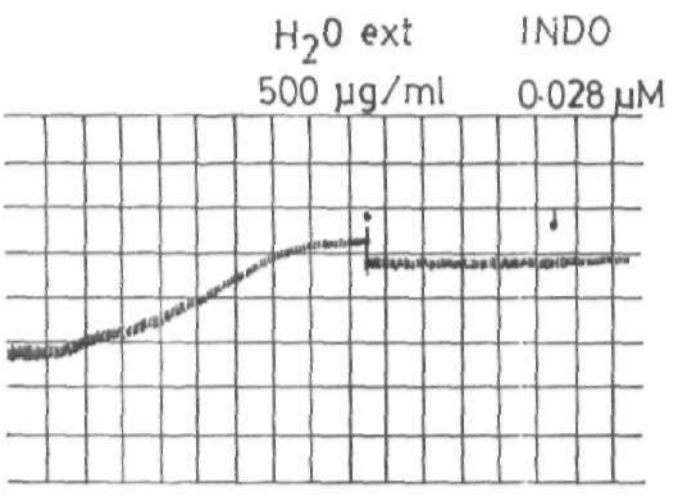
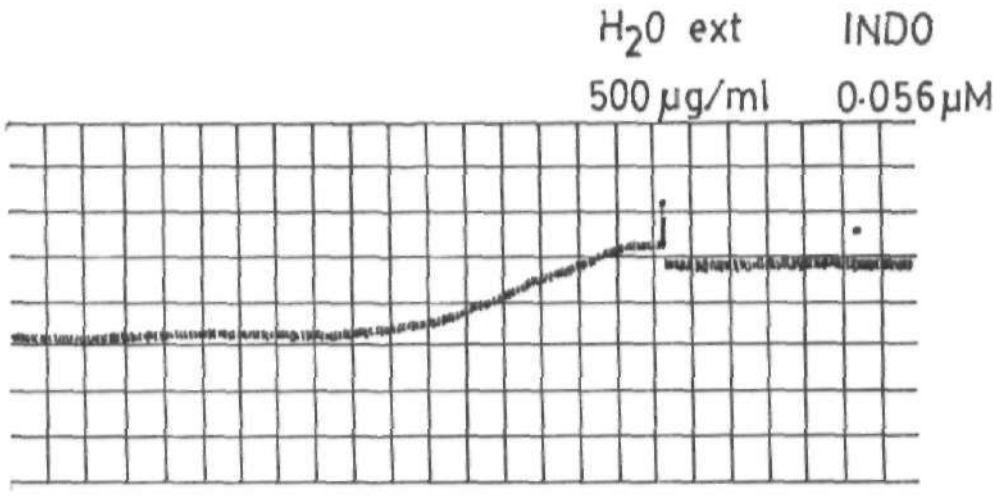


FIG. 3·3·24a: Effect of Indomethacin (0·014-0·028 µM) on water extract- induced platelet aggregation in PRP



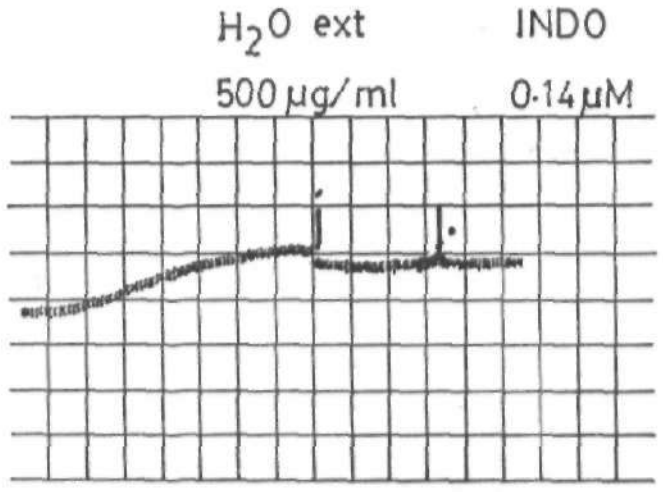


FIG. 3·3·24b Effect of Indomethacin (0·056--0·14µM) on water extract-induced platelet aggregation in PRP

3.3.3 Effect of E hirta leaf extracts on cat blood pressure and the isolated guinea pig atrium

A. Cat blood pressure

The mean arterial blood pressure (MABP) was 120 mmHg. The crude water and ethanol extracts of E.hirta, acetylcholine and choline lowered the blood pressure of the cat. Intravenous administration of the water extract (0.05 - 5mg/kg) produced dose-dependent decrease in MABP (Table 3.3.3.1); a typical tracing of which is presented in Fig. 3.3.3.1. The EC_{50} value of the water extract was 0.12 $\pm$ 0.02 mg/kg. Water extract produced a maximum fall in MABP of 100 mmHg at a dose of 1 mg/kg. Intravenous administration of the ethanol extract (0.5 - 10 mg/kg) also produced dose dependent decrease in MABP (Table 3.3.3.2). The EC_{50} value for ethanol extract was 1.5 $\pm$ 0.11 mg/kg. Ethanol extract produced a maximum fall in MABP of 100 mmHg at a dose of 5 mg/kg. Intravenous administration of acetylcholine (0.005 - 0.5μg/kg) produced dose-dependent decrease in MABP (Table 3.3.3.3). The EC_{50} value for acetylcholine was 0.009 $\pm$ 8.1 x 10^{-4} μg/kg. Acetylcholine produced a maximum fall in MABP of 98.5 mmHg at a dose of 0.05 μg/kg (Table 3.3.3.3). A typical tracing is presented in Fig. 3.3.3.2. Intravenous administration of choline (0.5 - 1mg\kg) produced dose-dependent decrease in MABP (results not shown).

Pretreatment of the cat with atropine (5.0 and 10 μg/kg) reduced the effects of both extracts and acetylcholine on MABP (Fig. 3.3.3 :- 3.4.5.6.7). There was a shift to the right, though this was not a parallel shift as expected for competitive antagonism. Increase in the doses of the extract, acetylcholine and choline that produced maximum fall in MABP (ie. asymptote) in the absence of atropine never produced maximum fall in MABP in the presence of atropine. The lower dose of atropine (5 μg/kg) reduced the maximum fall in MABP; for water extract (5 mg/kg) from 100 mmHg to 75.6 $\pm$ 3.04 mmHg (Table 3.3.3.1); for ethanol extract (10 mg/kg) from 100 mmHg to 16.7 $\pm$ 2.56 mmHg (Table 3.3.3.2) and for

acetylcholine (0.5 μg/kg) from 98.5 $\pm$ 0.14 mmHg to 88.5 $\pm$ 0.61 mmHg (Table 3.3.3.3). However, pretreatment with atropine (10 μg/kg)further reduced the maximum fall in MABP; for water extract (5 mg/kg) from 100 mmHg to 57.5 $\pm$ 2.41 mmHg (Table 3.3.3.1); for ethanol extract (10 mg/kg) from 100 mmHg to 8.6 $\pm$ 1.43 (Table 3.3.3.2) and for acetylcholine (0.5 μg/kg) from 98.5 $\pm$ 0.14 mmHg to 59.3 $\pm$ 2.4 mmHg (Table 3.3.3.3).

The EC_{50} value for water extract changed from 0.12 $\pm$ 0.02 mg/kg in the absence of atropine to 1.25 $\pm$ 0.03 mg/kg in the presence of atropine (5 μg/kg) and 1.98 $\pm$ 0.06 mg/kg (with 10 μg/kg). The EC_{50} for ethanol extract was 1.5 $\pm$ 0.11 mg/kg in the absence of atropine and after pretreatment with atropine, it never produced 50% maximum fall in MABP despite the increased dose. The EC_{50} value of acetylcholine shifted from 0.009 $\pm$ 8.1 x 10^{-4} μg/kg in the absence of atropine to 0.01 $\pm$ 1.4 x 10^{-3} μg/kg in the presence of atropine (5 μg/kg) and 0.018 $\pm$ 1.1 x 10^{-4} μg/kg (in 10 μg/kg). The water extract (0.5 mg/kg) produced the same percent maximum fall in MABP as ethanol (10mg/kg), therefore it has a higher hypotensive effect at a lower dose than the ethanol extract, thus is more potent (Fig. 3.3.3.9a). Propranolol (1 μM) injected 2 minutes before the acetylcholine or the extracts did not reduce the depressor effect of the latter significantly (Data not shown).

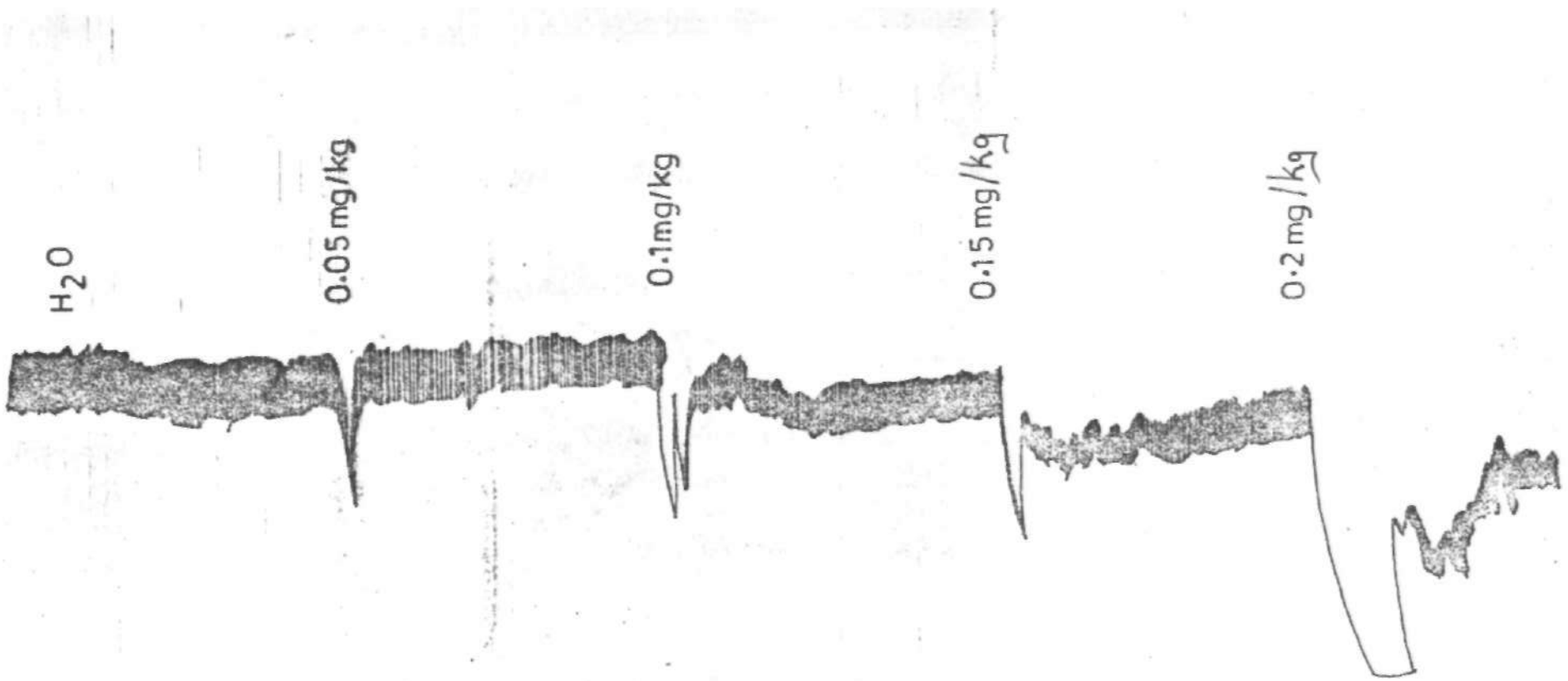


Fig. 3.3.3.1. Dose-related effect of Water extract of Euphorbia hirta leaves on cat Blood pressure.

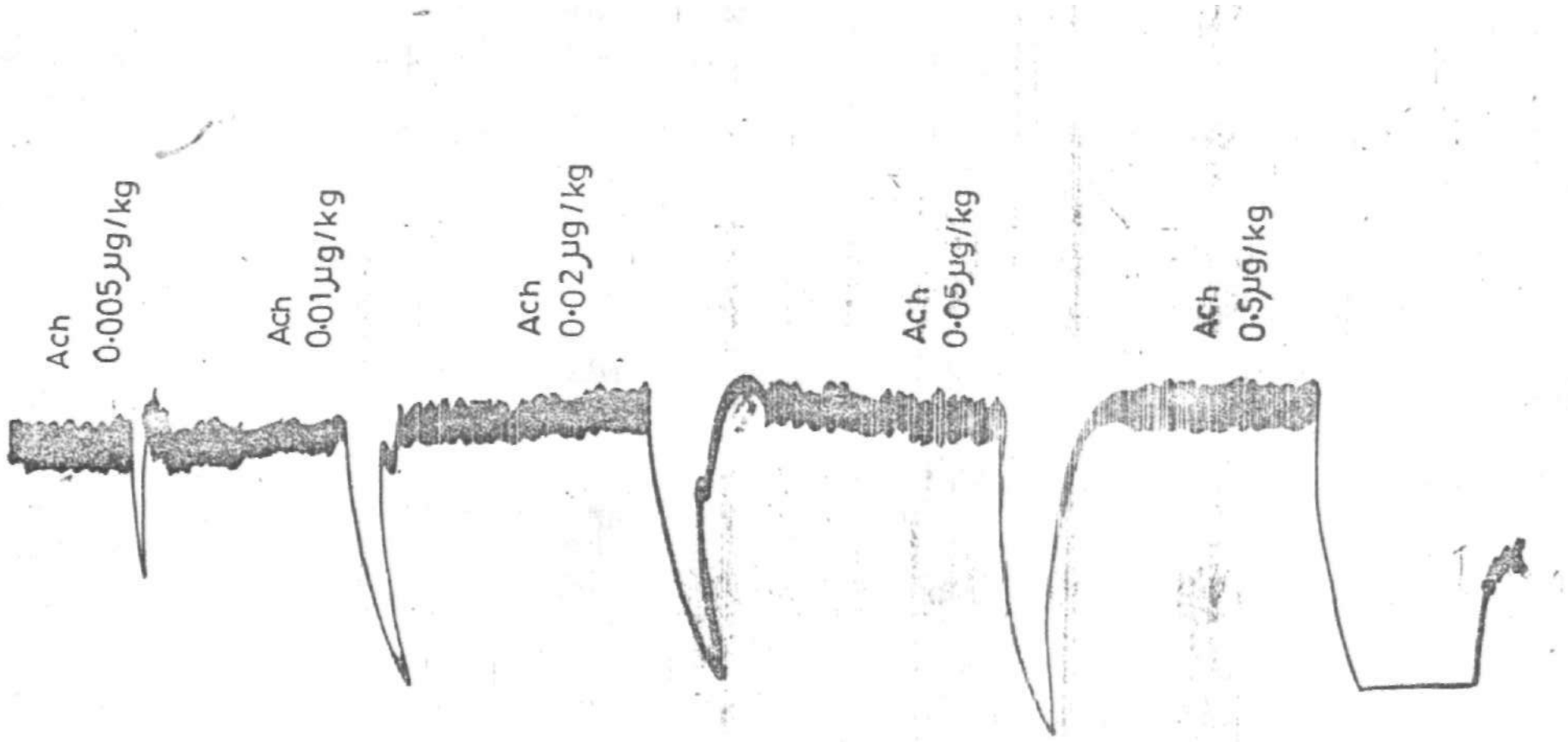


Fig.3.3.3.2. -Dose - related effect of Acetylcholine on cat Blood Pressure.

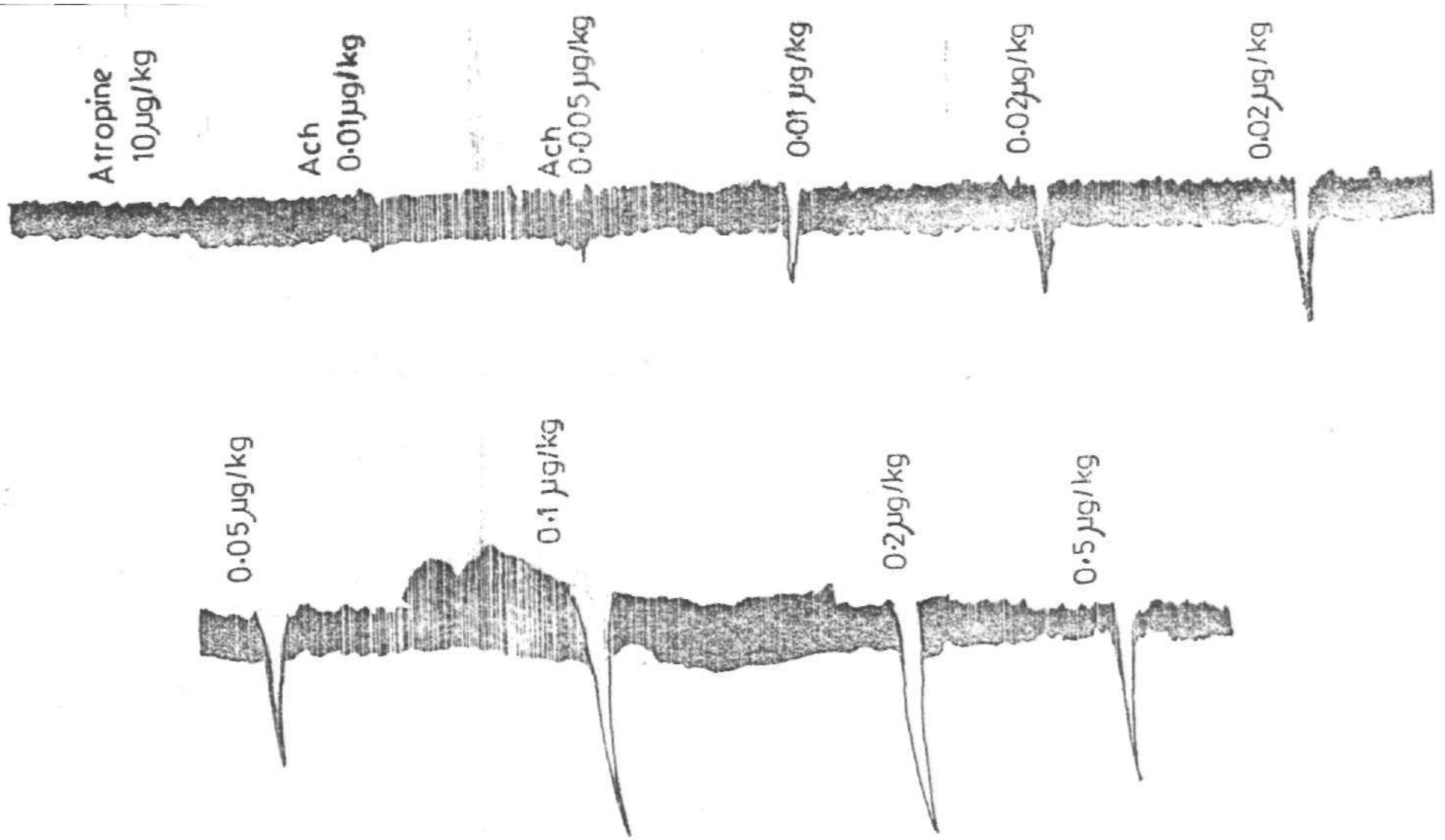


Fig.3.3.3.3.-Dose-related effect of Acetylcholine on cat Blood pressure in the presence of Atropine.

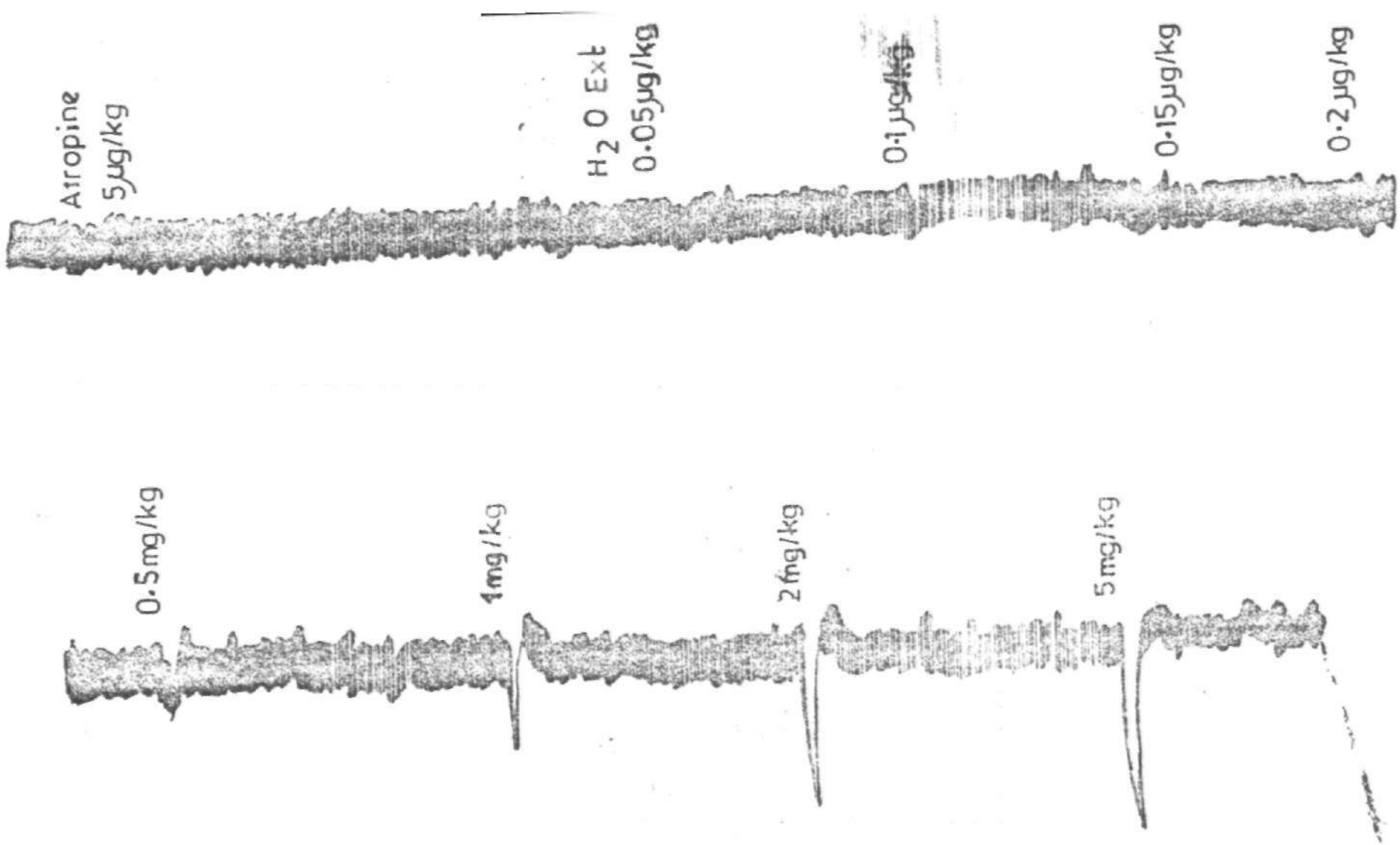
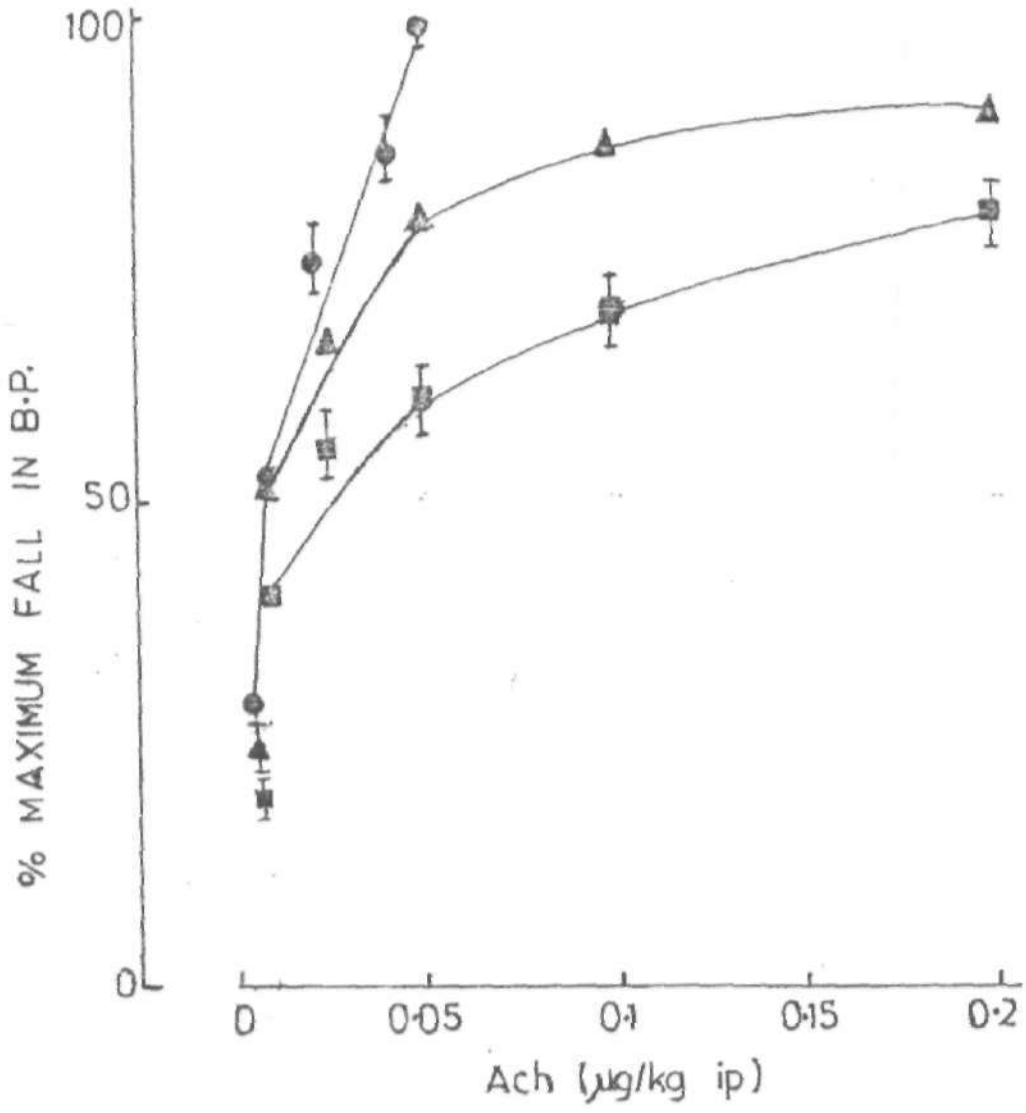


Fig.3.3.3.4. Dose-related effect of Water extract of Euphorbia hirta leaves on cat blood pressure in the presence of Atropine.



ig.3.3.3.5. Effect of acetylcholine on cat Bp in the absence (●—●) and presence of atropine 5 µg/Kg (▲—▲) and 10 µg/kg (■—■). Results are means of 2 experiments.

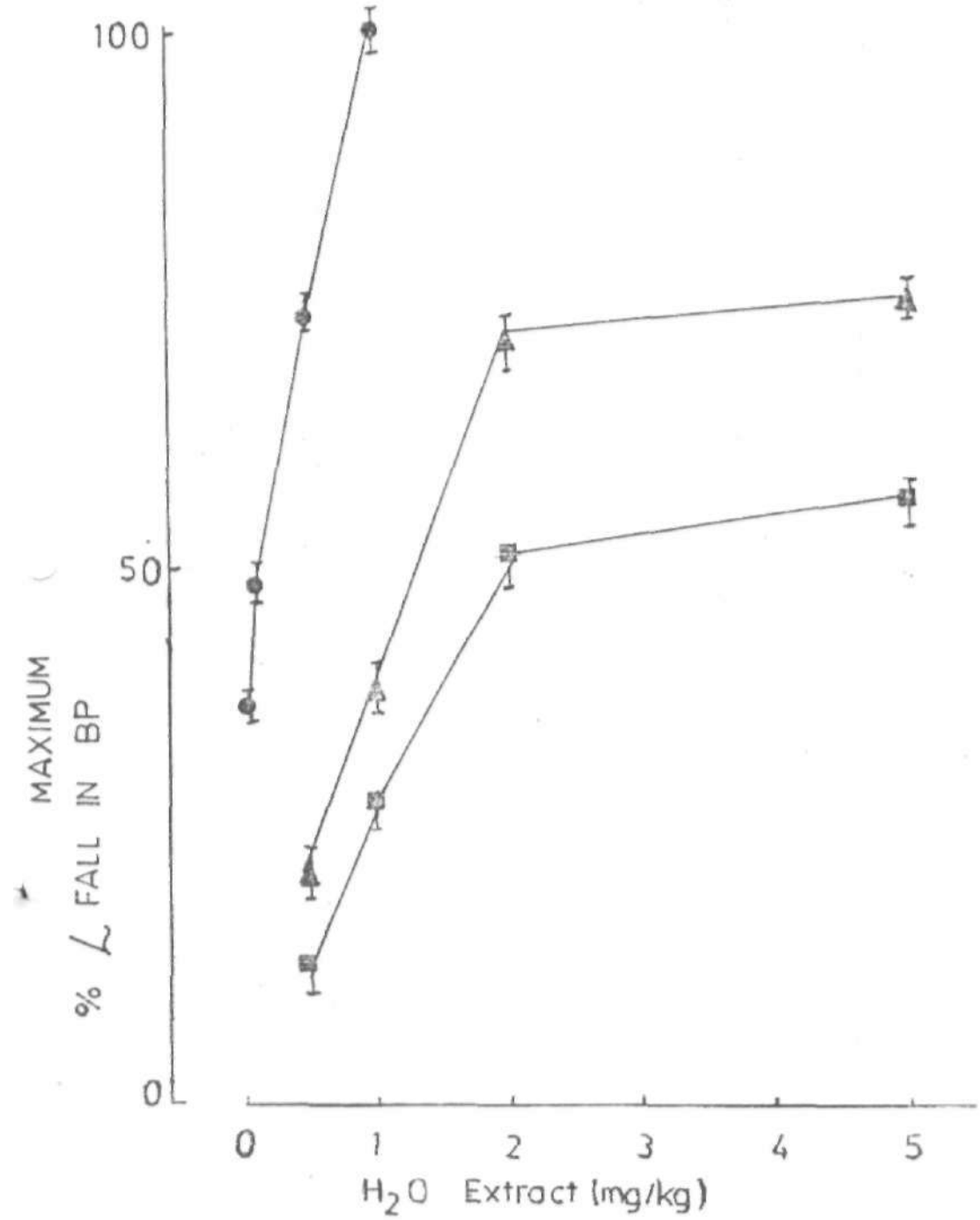


Fig. 3.3.3.6. Effect of water extract on cat BP in the absence (●—●) and presence of Atropine 5 µg/kg (▲—▲) and 10 µg/kg (■—■). Results are means of 2 experiments.

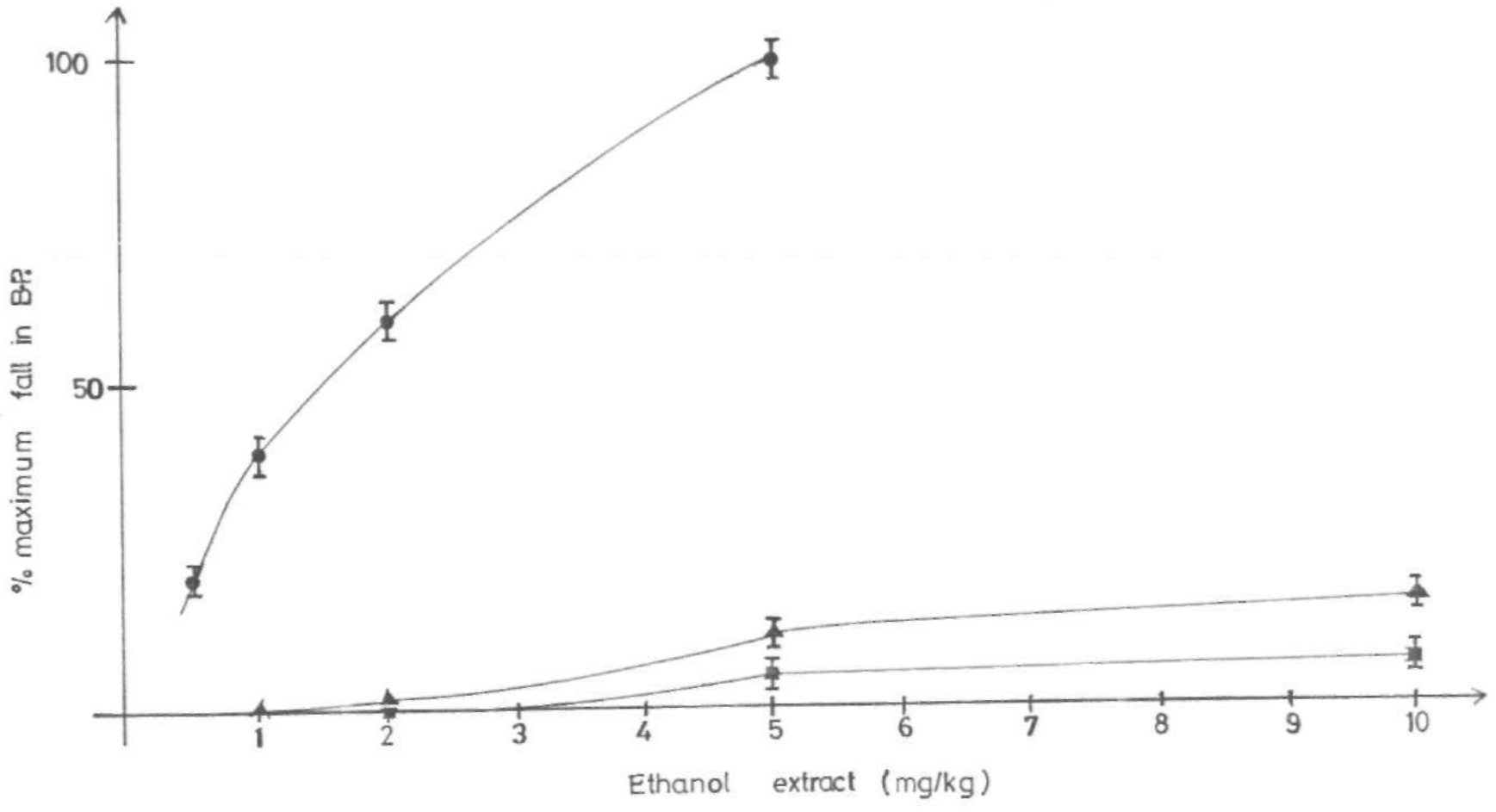


Fig 3·3·3·7

Effect of ethanol extract on Cat B.P. in the absence (●—●) and presence (▲—▲) of Atropine (5µg/kg) and 10µg/kg (■—■)
Results are means of two experiments

Table 3.3.3.1: The effect of water extract of E.hirta on cat B.P. in the absence and presence of atropine

Dose of H_2O extract (mg/kg)	% Maximum fall in MABP		
	Before atropine	After atropine	
		5μg/kg	10μg/kg
0.05	37.5 ± 0.31	0.0	0.0
0.10	48.8 ± 0.73	0.0	0.0
0.15	53.8 ± 0.43	0.0	0.0
0.20	56.8 ± 1.01	0.0	0.0
0.50	73.8 ± 1.04	21.9 ± 0.14	13.8 ± 0.16
1.00	100	38.8 ± 0.16	28.8 ± 1.30
2.00	100	70.6 ± 2.19	51.3 ± 0.87
5.00	100	75.6 ± 3.04	57.5 ± 2.41

Table 3.3.3.2: The effect of ethanol extract of E.hirta on cat B.P. in the absence and presence of atropine

Dose of EtOH extract (mg/kg)	% Maximum fall in MABP		
	Before atropine	After atropine	
		5μg/kg	10μg/kg
0.5	19.8 ± 1.01	0	0
1.0	40.0 ± 1.36	0	0
2.0	60.0 ± 0.71	2.0 ± 0.98	0
5.0	100	11.9 ± 3.11	5.3 ± 0.68
10.0	100	16.7 ± 2.56	8.6 ± 1.43

Table 3.3.3.3: The effects of acetylcholine on cat B.P. in the absence and presence of atropine

Dose of H_2O extract (mg/kg)	% Maximum fall in MABP		
	Before atropine	After atropine	
		5μg/kg	10μg/kg
0.005	28.5 ± 1.11	23.8 ± 0.79	19.3 ± 0.77
0.01	52.4 ± 2.77	50.0 ± 3.08	40.0 ± 1.43
0.02	74.6 ± 3.69	65.4 ± 3.14	55.4 ± 0.16
0.05	98.5 ± 0.14	78.5 ± 2.04	59.3 ± 2.40
0.10	100	85.4 ± 1.16	68.5 ± 3.17
0.20	100	88.5 ± 0.61	79.3 ± 0.39
0.50	100	-	-

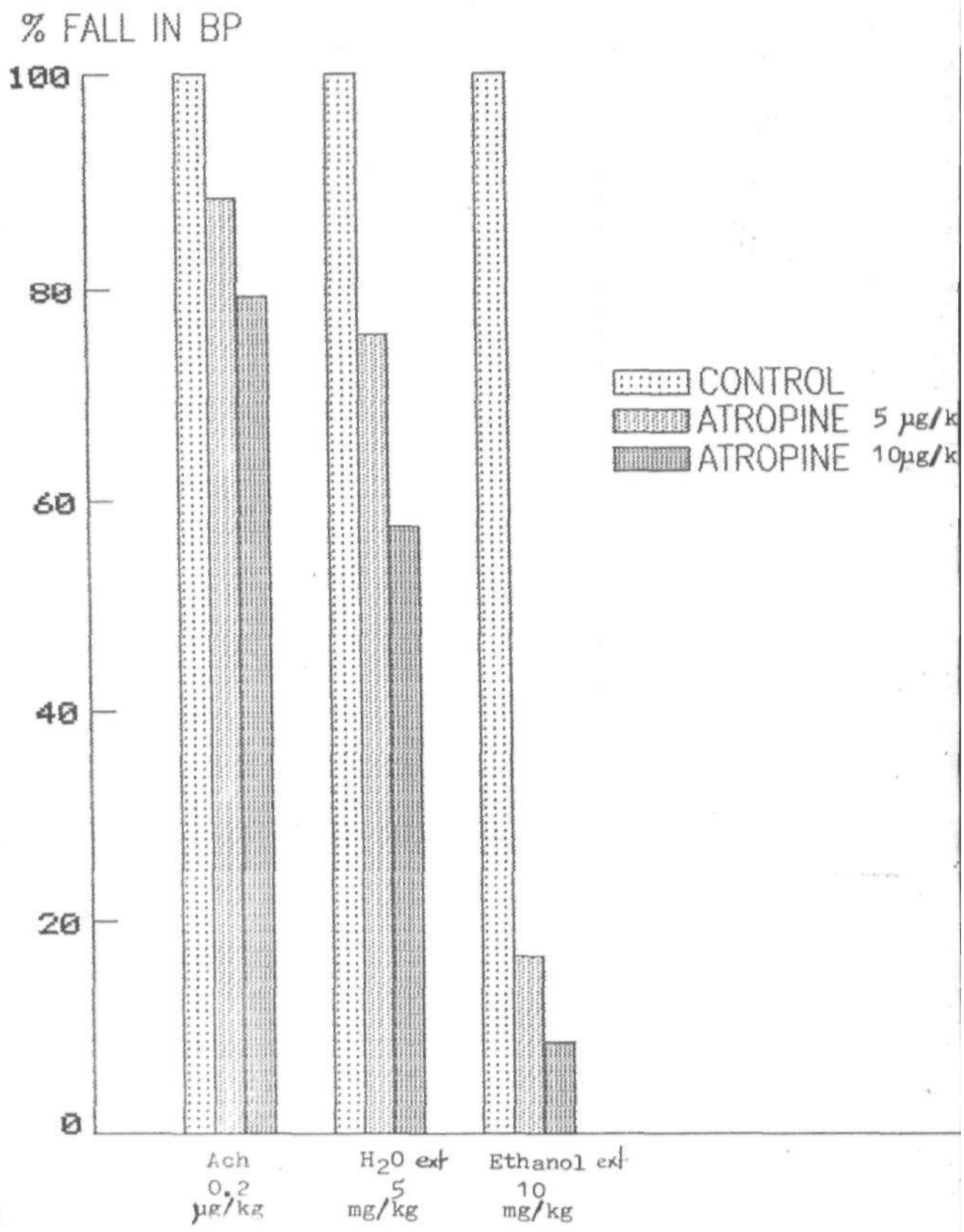


Fig. 3.3.3.9a. Dose related effect of atropine on the depressor action of acetylcholine, water and ethanol extracts of E. hirta on cat B.P.

B Mechanism of E.hirta leaf extract-induced fall in MABP

Investigation of effect of E. hirta leaf extract on muscarinic subtypes

The effect of E.hirta leaf extracts in the absence and presence of a non-selective muscarinic antagonist like atropine showed significant changes, which cannot be overlooked. Therefore, the effect of other specific muscarinic receptor antagonists on the extracts was investigated. The water extract was used as this is the most common form of administration in traditional medicine.

C Effect of E.hirta on M_1 muscarinic receptor subtype using an anaesthetized cat

The mean arterial blood pressure (MABP) was still 120 mmHg. The effect of the crude water extract of E.hirta, acetylcholine and choline were investigated in the presence of pirenzepine - an M_1 subtype antagonist. The M_1 receptor is found in the cerebral cortexand smooth muscles. Identical doses of water extract, acetylcholine and choline that gave maximal fall in blood pressure (from 3.3.3A) were repeated in the absence and presence of pirenzepine (5 mg/kg). Water extract produced a maximum fall in MABP of 100% at a dose of 1mg/kg. Acetylcholine and choline produced maximum fall in MABP of 100% at 0.05 μg/kg and 0.5 mg/kg respectively.

Pretreatment of the cat with pirenzepine (5 mg/kg) reduced the effects of the leaf extract, acetylcholine and choline on MABP (Fig 3.3.3.9b). Pirenzepine reduced the maximum fall in MABP from 100% to 29% for water extract; for acetylcholine from 100% to 67% and for choline from 100% to 78%, respectively. The percentage blockade in the presence of pirenzepine was 71% for E.hirta water extract, 22% for acetylcholine and 33% for choline.

D Effect of E.hirta on M_2 muscarinic receptor subtype using the isolated guinea pig atrium

The arterial blood pressure depends on the cardiac output and the totalperipheral resistance. Therefore changes in the blood pressure (BP) can be brought about by alteration in cardiac output (CO), total peripheral resistance (TPR) or both. Other factors that affect the arterial BP are elasticity of the arteries, blood volume, viscosity of the blood and force of contraction (Tarverner, 1975). The specific muscarinic receptor in the heart is M_2 subtype. The effects of water extract, acetylcholine and choline on the force of contraction of the myocardium were evaluated based on the % relaxations produced on the isolated guinea pig atrium.

The crude water extract of E.hirta, acetylcholine and choline produced dose-dependent relaxations on the isolated atrium. The EC_{50} for acetylcholine was $0.125 \pm 1.04 \times 10^{-3}$ μg/ml, for choline $0.05 \pm 1.1 \times 10^{-3}$ mg/ml and water extract was 1.85 ± 0.86 mg/ml (Table 3.3.3.4). The water extract was not as potent as choline which was in turn less potent than acetylcholine. In the presence of methoctramine (0.6 μM) - which is a specific M_2 antagonist, there was partial blockade of the effects of acetylcholine (0.5 μg/ml) - 33.4%, choline (0.25 mg/ml) - 38.1% and water extract (2.5 mg/ml) which showed the most blockade 87.9% (Table 3.3.3.5 Fig. 3.3.3.8 and Fig. 3.3.3.9c).

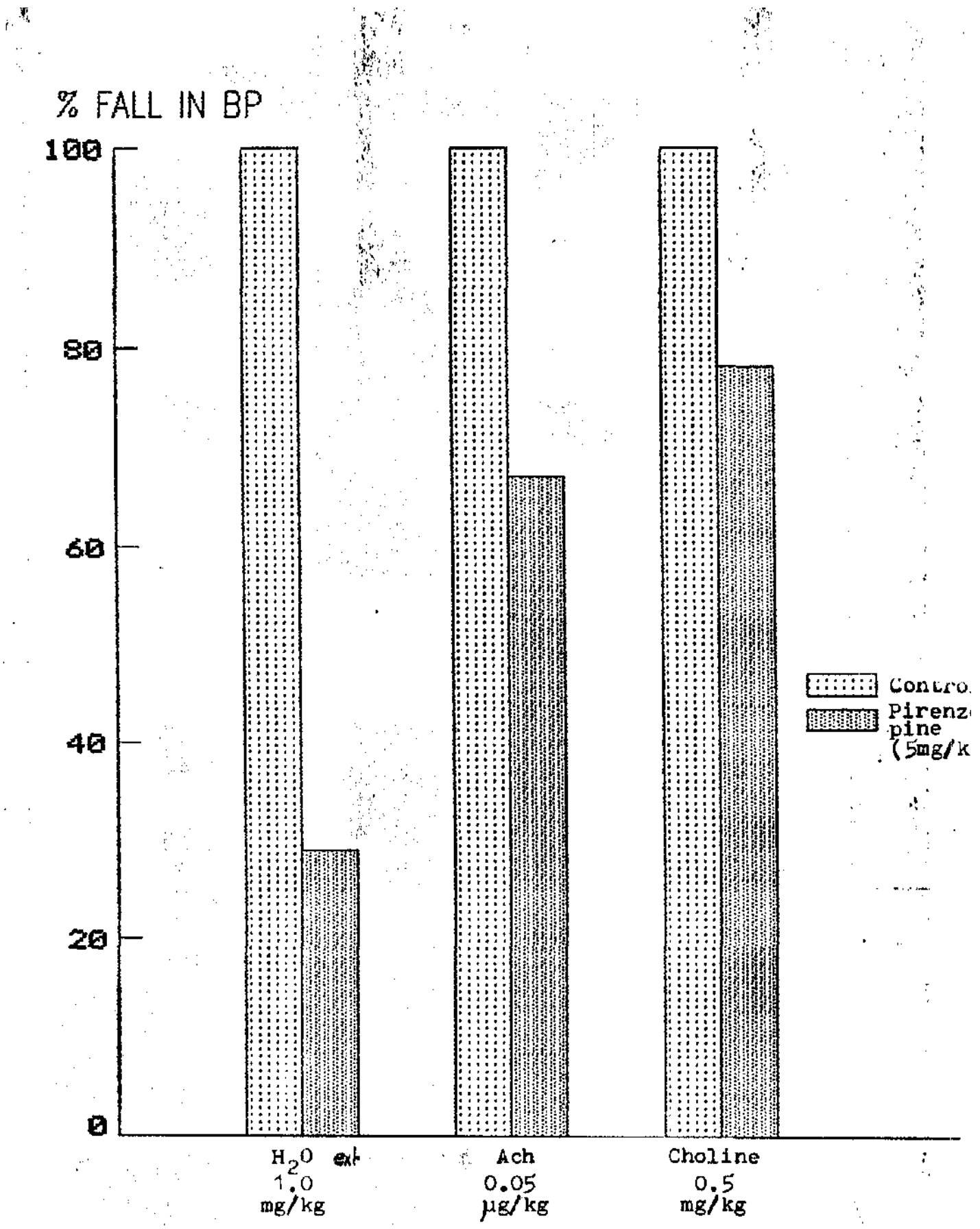


Fig. 3.3.3.9b. Dose related effect of pirenzepine (5 mg/kg) on the depressor action of E. hirta water extract, acetylcholine and choline on cat B.P.

Table 3.3.3.4: The relaxant effect of acetylcholine, choline and water extract on the isolated guinea pig atrium

	Concentration	Average % relaxation (cm)
Ach	0.025 μg/ml	14.3 ± 3.13
	0.05 μg/ml	28.6 ± 1.71
	0.125 μg/ml	50.0 ± 0.63
	0.25 μg/ml	85.7 ± 0.79
	0.5 μg/ml	93.8 ± 0.11
Choline	0.025 mg/ml	33.3 ± 1.37
	0.05 mg/ml	50.0 ± 1.46
	0.25 mg/ml	90.9 ± 0.91
H_2O extract	0.25 mg/ml	8.3 ± 1.01
	0.50 mg/ml	9.1 ± 2.17
	1.00 mg/ml	25.0 ± 2.63
	1.50 mg/ml	44.4 ± 3.84
	2.50 mg/ml	63.6 ± 3.16

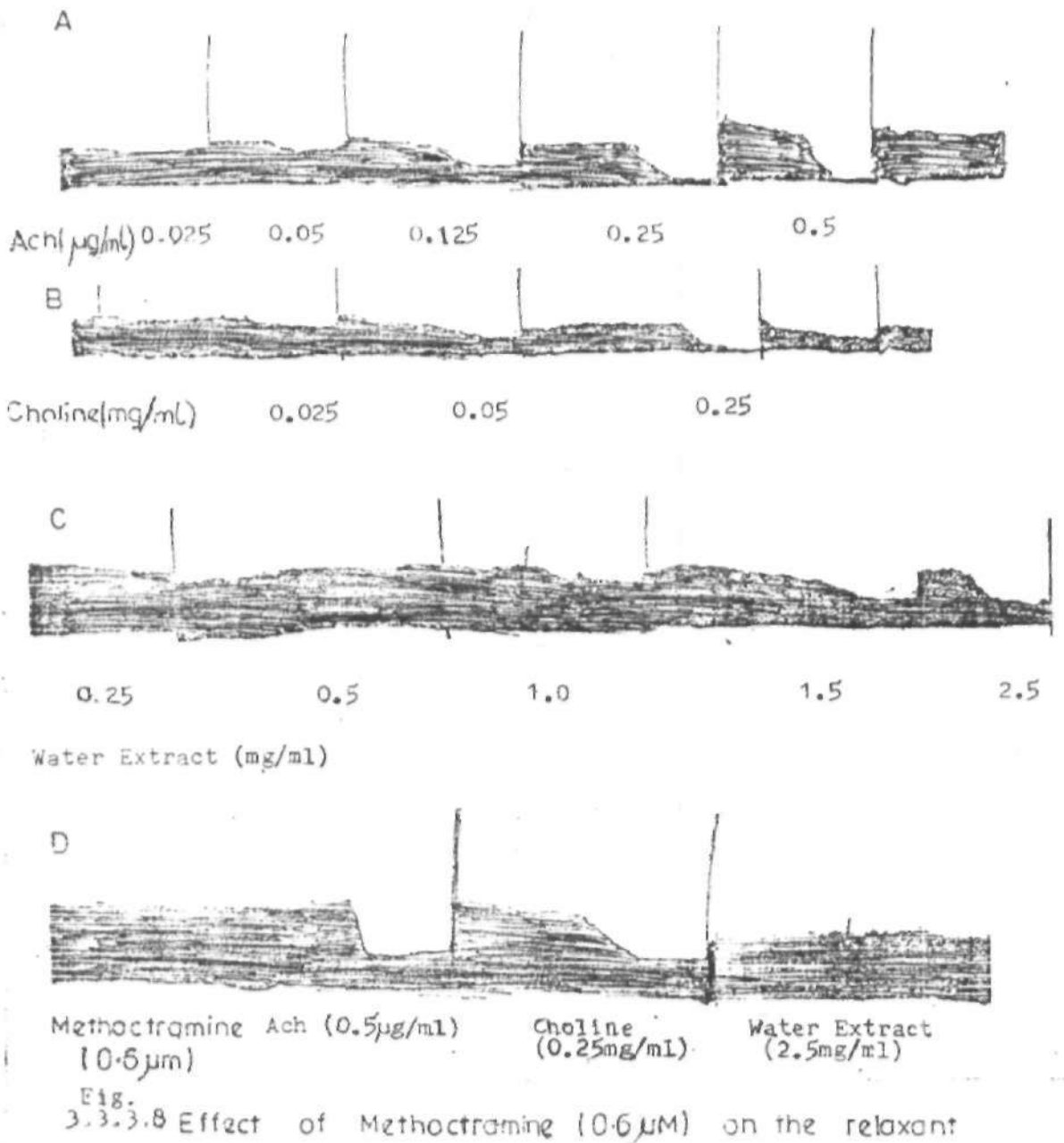


Fig. 3.3.3.8 Effect of Methoctramine (0·6μM) on the relaxant effects of Ach, choline and Water extract of E. hirta leaves on guinea pig isolated atrium.

Table 3.3.3.5: The effects of acetylcholine, choline and water extracts on isolated guinea pig atrium in the absence and presence of methoctramine (0.6 μm)

	Average % relaxations		% Blockade
	Before methotramine	After methotramine	
Ach (0.5μg/ml)	93.8 $\pm$ 0.11	62.5 $\pm$ 3.31	33.4
Choline (0.25mg/ml)	90.9 $\pm$ 0.91	56.3 $\pm$ 1.79	38.1
H_20 extract (2.5mg/ml)	63.6 $\pm$ 3.16	7.7 $\pm$ 2.66	87.9

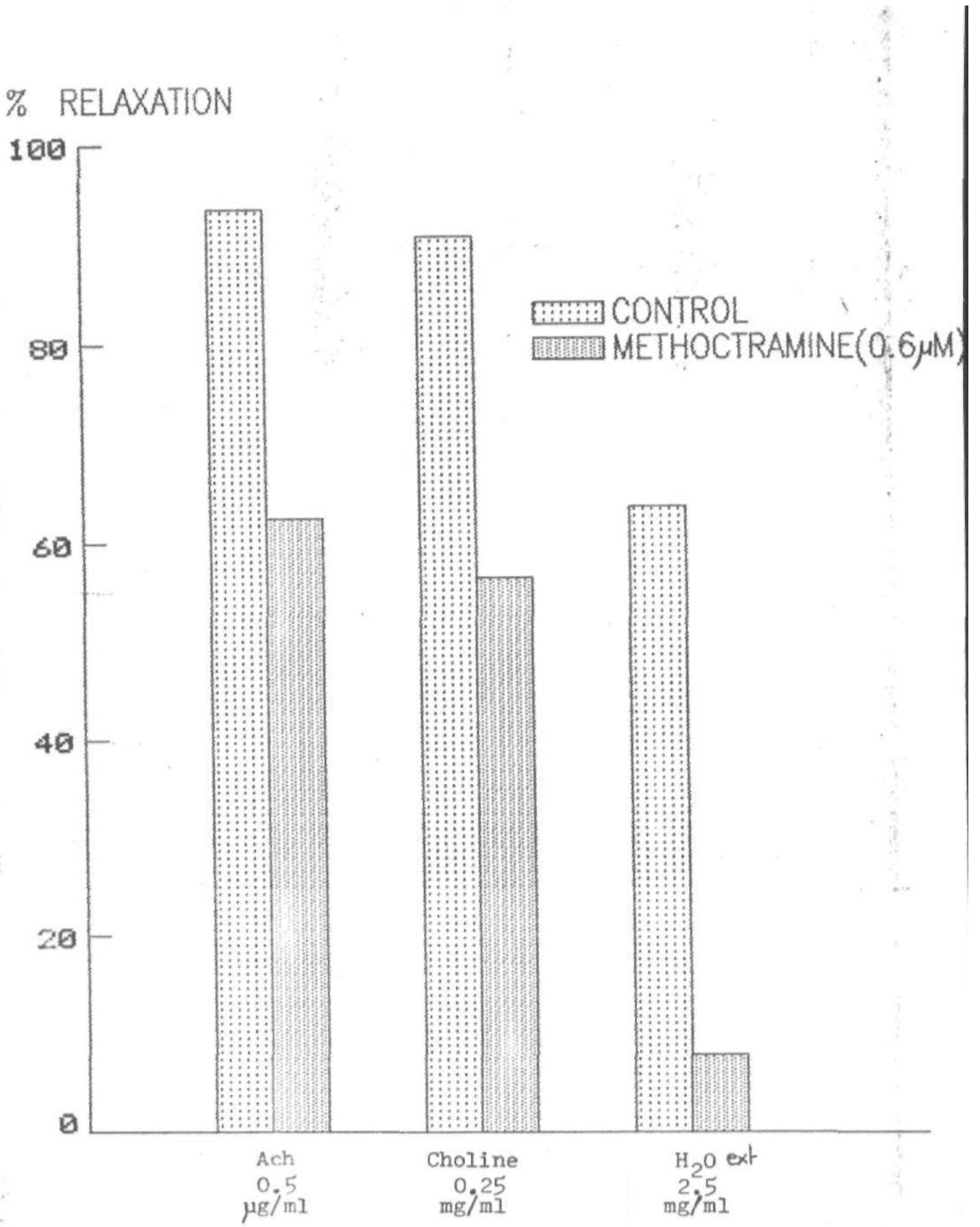


Fig. 3.3.3.9c. Dose related effect of Methoctramine (0.6 μM) on the relaxant action of acetylcholine, choline and water extract of E. hirta on isolated guinea-pig atrium.

3.3.4 Effect of E. hirta leaf extracts on urine output and urinary electrolytes

The results of screening the water and ethanol extracts of the leaves of E.hirta for diuresis are shown in Table 3.3.4.1. The water extract showed little change in urine output (U.O) at the two doses employed (at 50 mg/kg, UO was 3.9ml; at 100mg/kg UO was 4.08ml after the 24h period.). However, for 100 mg/kg, urine output was significant at 6h, when compared to the control; whilst for 50mg/kg urine output was significant at 12h and this continued through the 24h experimental period ($p < 0.05$). Diuresis was induced after 1h with the higher dose (100 mg/kg) of water extract; this activity was minimal when compared to that obtained at the lower dose (50mg/kg) which induced diuresis after 3h (Fig 3.3.4.1). The diuretic effect of ethanol extract was significant at the lower dose (50 mg/kg) from 6h (UO was 13.9ml) ($p < 0.05$), however for 100 mg/kg it was only significant at 24h which shows little or no diuresis. Diuresis by the ethanol extract was induced at both doses after the first 3h (Fig. 3.3.4.2). Urine output increased gradually till 12h, a sudden rise occured between 12 and 24h and this is more prominent with the lower dose (50 mg/kg).

The control (saline) showed only slight increase in urine output within the 24 experimental hours (Table 3.3.4.1). Frusemide (5 mg/kg) induced a brisk and significant diuresis within 30 minutes of administration. This reached a maximum in 3h and stayed constant until about 12h, then increased gradually till 24h. (Fig. 3.3.4.3). Acetazolamide (5 mg/kg) induced diuresis within 30 min which gradually increased within the 24h experimental period (Fig. 3.3.4.4). However, diuresis was only statistically significant after 1h. Water extract (50 and 100mg/kg) increased the pH of the urine from 8.8 to 10 $\pm$ 1.46, and 8.8 to 9.0 $\pm$ 1.4 respectively. The ethanol extracts increased urine pH from 8.8 to 10.3 $\pm$ 0.49. Acetazolamide increased the urine pH from 8.8 to 10.1, thus making the urine in each case more alkaline. However frusemide (5mg/kg) had no significant effect on the urinary pH.

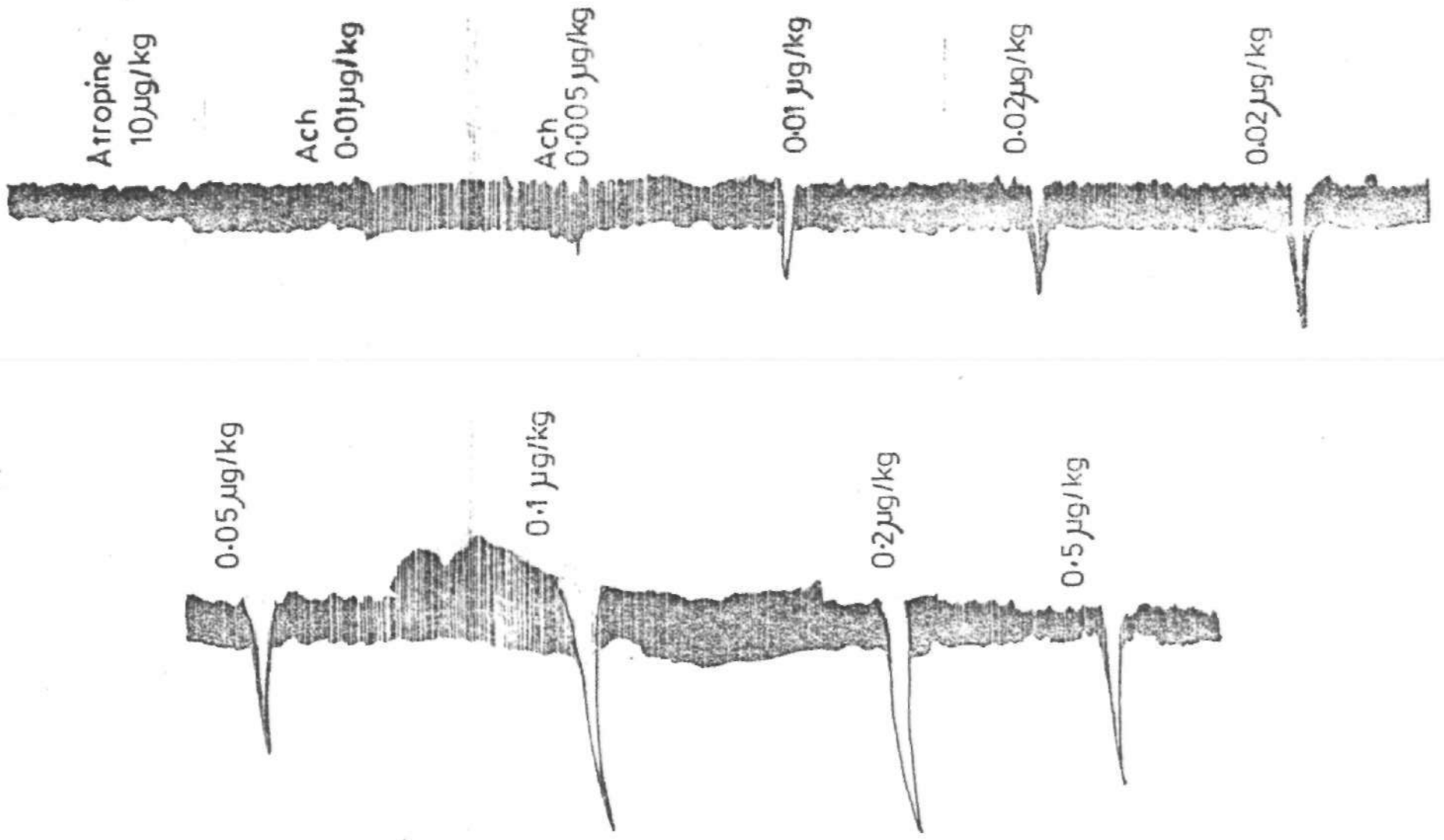


Fig.3.3.3.3.-Dose_related effect of Acetylcholine on cat Blood pressure in the presence of Atropine.

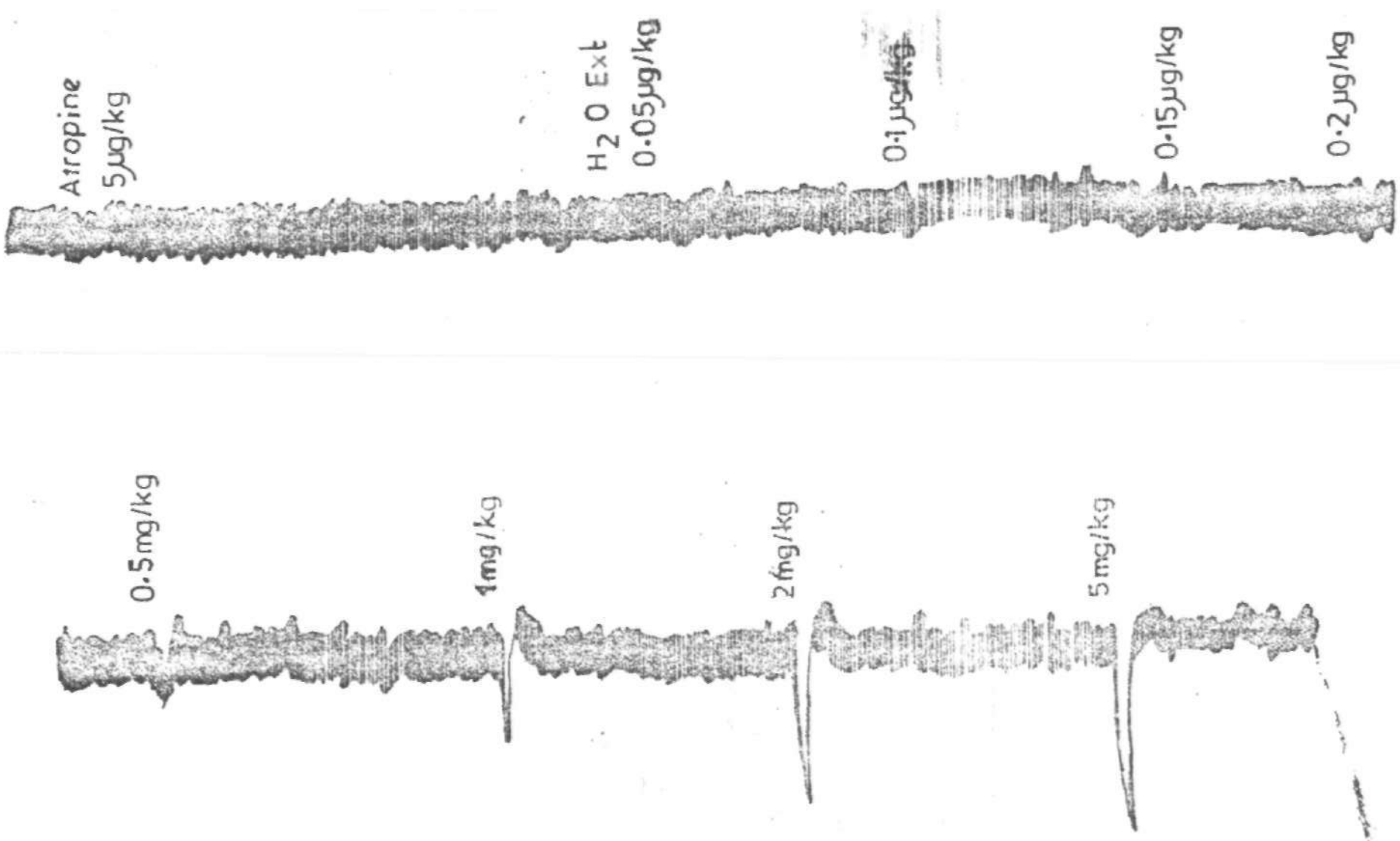


Fig.3.3.3.4. Dose-related effect of Water extract of Euphorbia hirta leaves on cat blood pressure in the presence of Atropine.

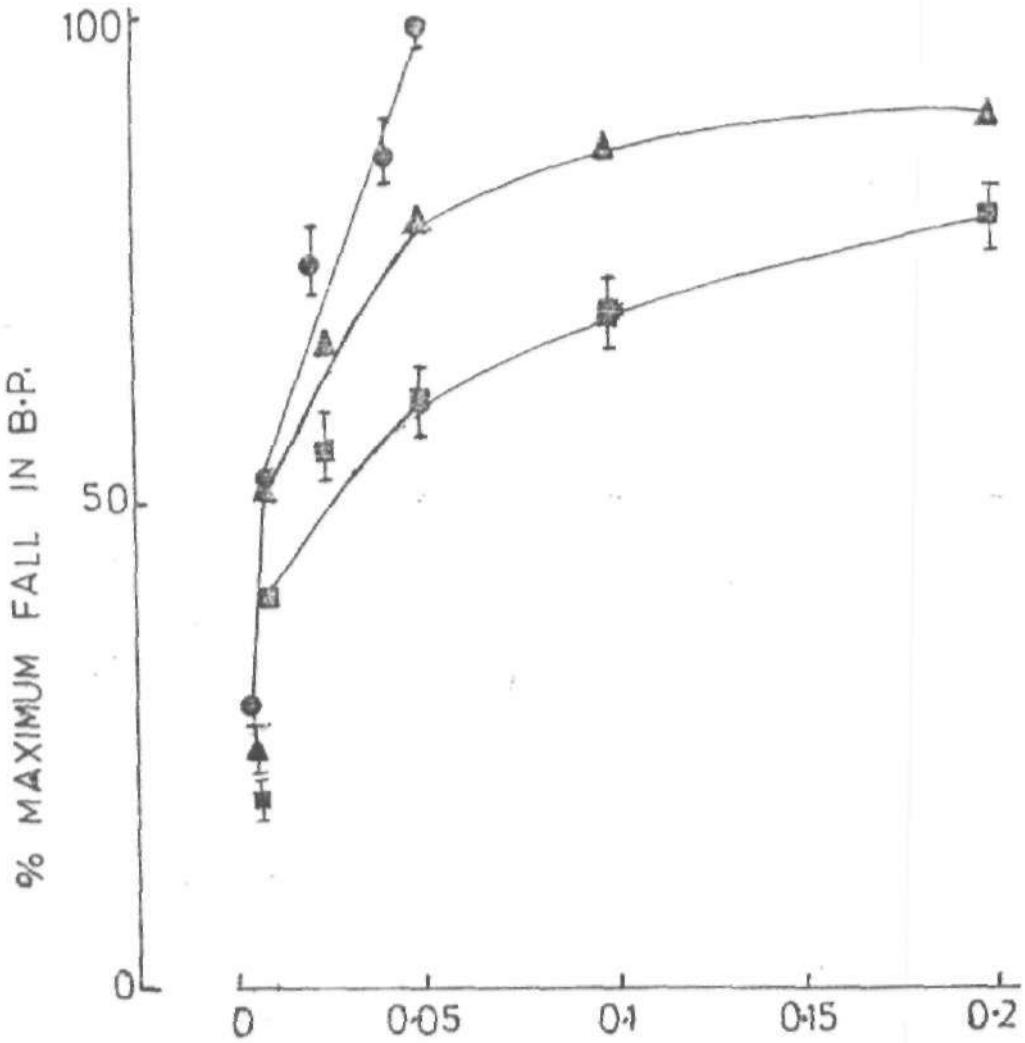


Fig.3.3.3.5. Effect of acetylcholine on cat Bp in the absence (●—●) and presence of atropine 5 µg/Kg (▲—▲) and 10 µg/kg (■—■). Results are means of 2 experiments.

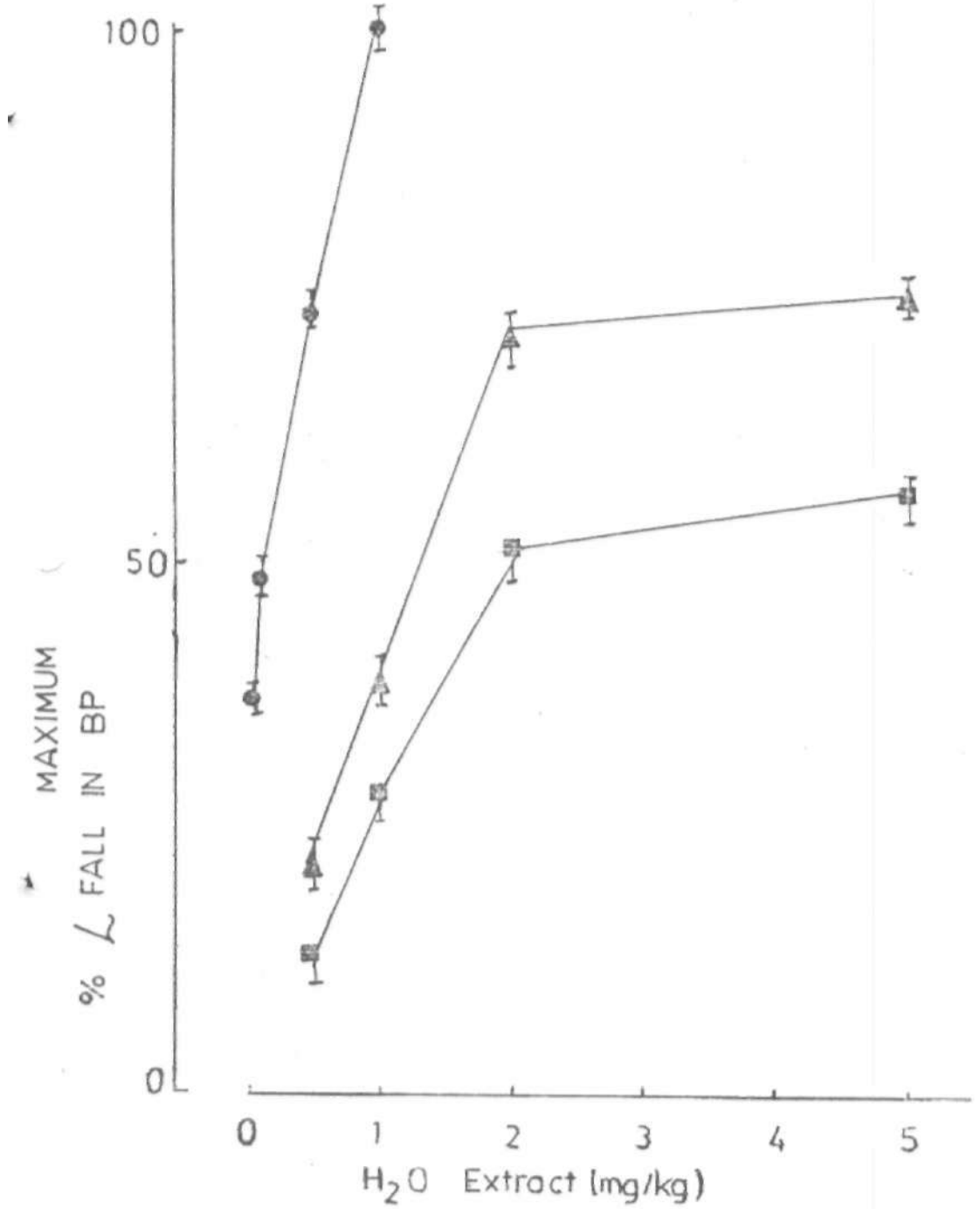


Fig. 3.3.3.6. Effect of water extract on cat BP in the absence(●—●) and presence of Atropine 5 µg/kg (▲—▲) and 10 µg/kg(■—■) Results are means of 2 experiments.

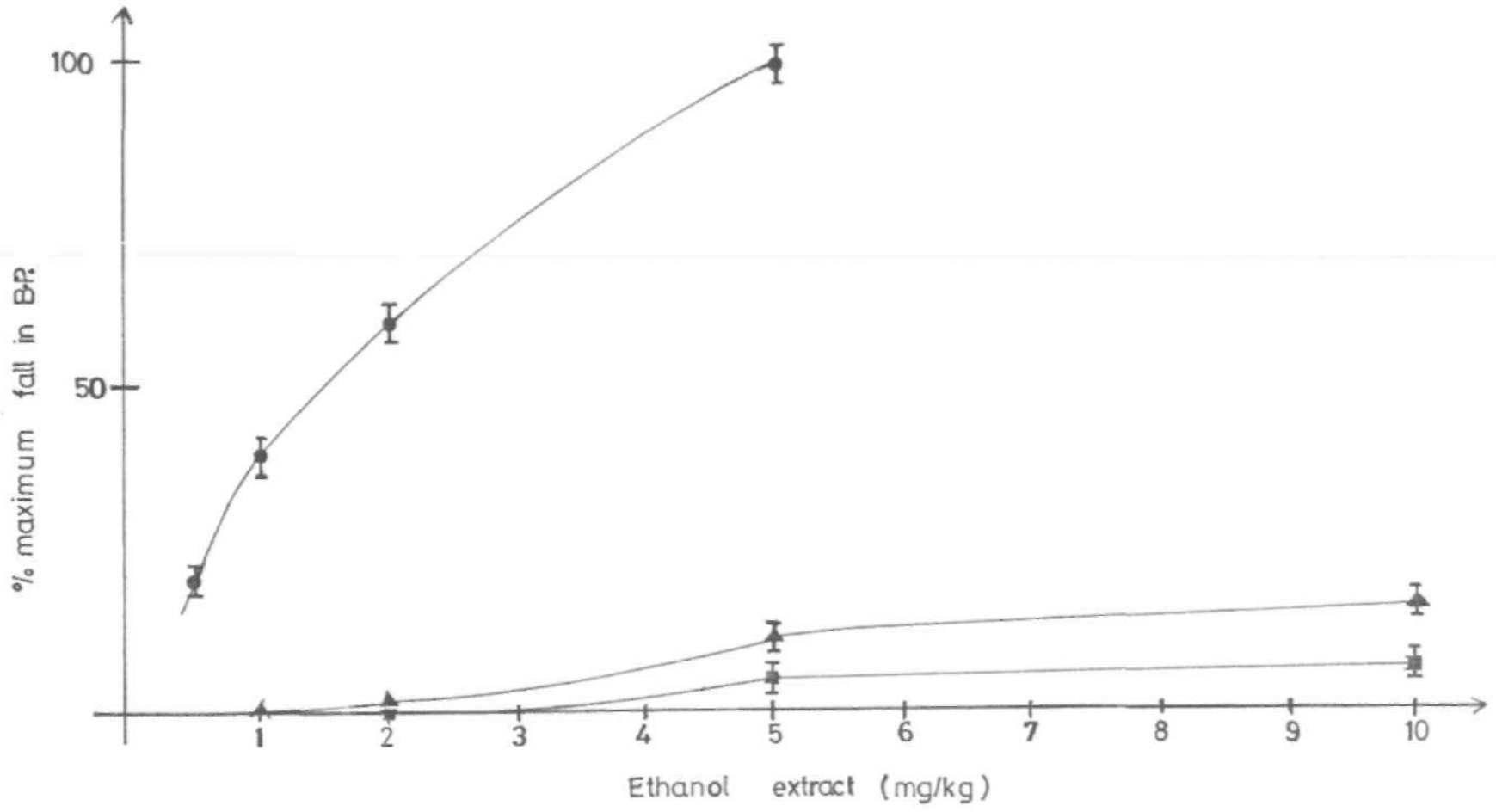


Fig 3·3·3·7

Effect of ethanol extract on Cat B.P. in the absence (●—●) and presence (▲—▲) of Atropine (5µg/kg) and 10µg/kg (■—■)
Results are means of two experiments

Table 3.3.3.1: The effect of water extract of E.hirta on cat B.P. in the absence and presence of atropine

Dose of H_2O extract (mg/kg)	% Maximum fall in MABP		
	Before atropine	After atropine	
		5μg/kg	10μg/kg
0.05	37.5 ± 0.31	0.0	0.0
0.10	48.8 ± 0.73	0.0	0.0
0.15	53.8 ± 0.43	0.0	0.0
0.20	56.8 ± 1.01	0.0	0.0
0.50	73.8 ± 1.04	21.9 ± 0.14	13.8 ± 0.16
1.00	100	38.8 ± 0.16	28.8 ± 1.30
2.00	100	70.6 ± 2.19	51.3 ± 0.87
5.00	100	75.6 ± 3.04	57.5 ± 2.41

Table 3.3.3.2: The effect of ethanol extract of E.hirta on cat B.P. in the absence and presence of atropine

Dose of EtOH extract (mg/kg)	% Maximum fall in MABP		
	Before atropine	After atropine	
		5μg/kg	10μg/kg
0.5	19.8 ± 1.01	0	0
1.0	40.0 ± 1.36	0	0
2.0	60.0 ± 0.71	2.0 ± 0.98	0
5.0	100	11.9 ± 3.11	5.3 ± 0.68
10.0	100	16.7 ± 2.56	8.6 ± 1.43

Table 3.3.3.3: The effects of acetylcholine on cat B.P. in the absence and presence of atropine

Dose of H_2O extract (mg/kg)	% Maximum fall in MABP		
	Before atropine	After atropine	
		5μg/kg	10μg/kg
0.005	28.5 ± 1.11	23.8 ± 0.79	19.3 ± 0.77
0.01	52.4 ± 2.77	50.0 ± 3.08	40.0 ± 1.43
0.02	74.6 ± 3.69	65.4 ± 3.14	55.4 ± 0.16
0.05	98.5 ± 0.14	78.5 ± 2.04	59.3 ± 2.40
0.10	100	85.4 ± 1.16	68.5 ± 3.17
0.20	100	88.5 ± 0.61	79.3 ± 0.39
0.50	100	-	-

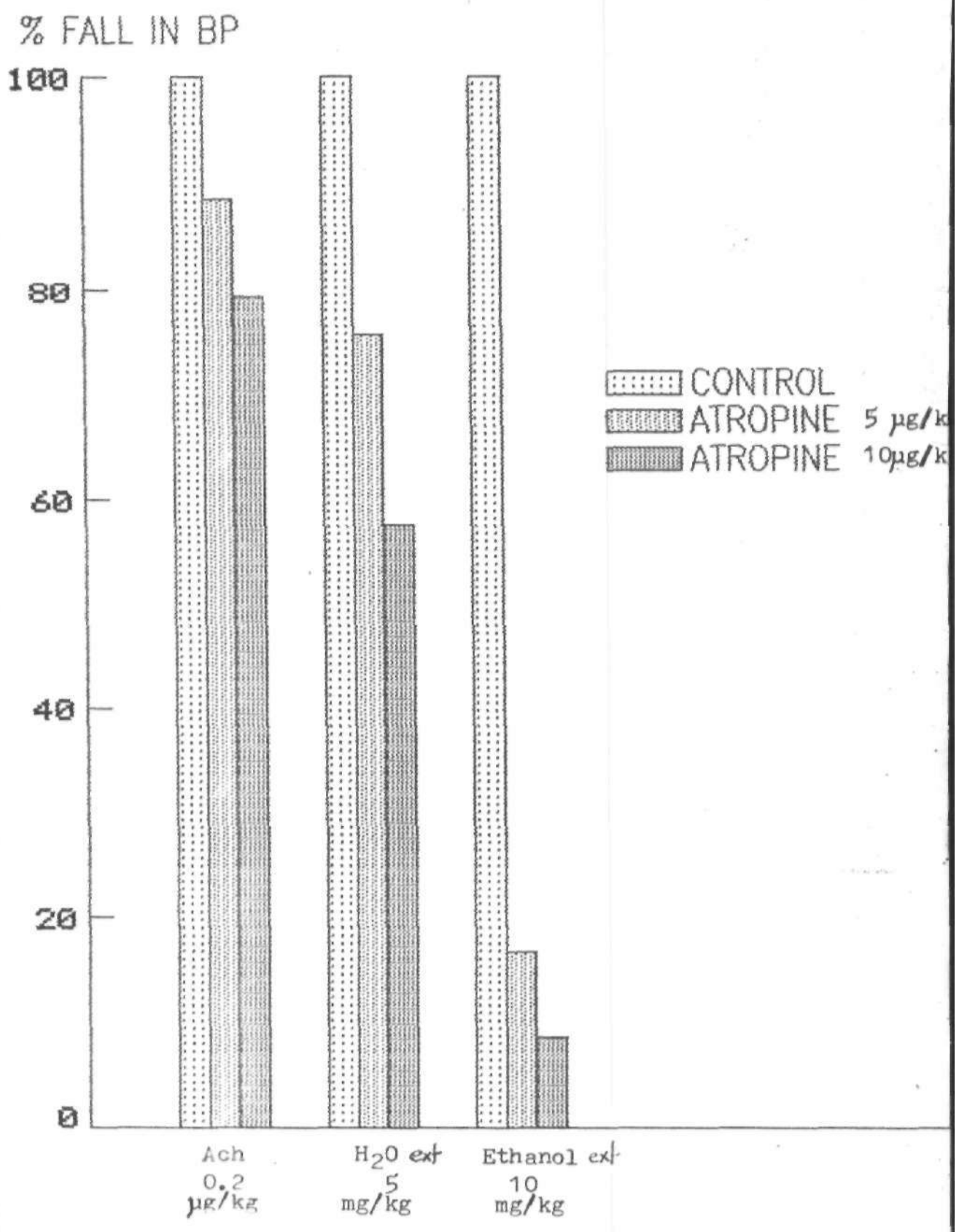


Fig. 3.3.3.9a. Dose related effect of atropine on the depressor action of acetylcholine, water and ethanol extracts of E. hirta on cat B.P.

B **Mechanism of E.hirta leaf extract-induced fall in MABP**

Investigation of effect of E. hirta leaf extract on muscarinic subtypes

The effect of E.hirta leaf extracts in the absence and presence of a non-selective muscarinic antagonist like atropine showed significant changes, which cannot be overlooked. Therefore, the effect of other specific muscarinic receptor antagonists on the extracts was investigated. The water extract was used as this is the most common form of administration in traditional medicine.

C **Effect of E.hirta on M_1 muscarinic receptor subtype using an anaesthetized cat**

The mean arterial blood pressure (MABP) was still 120 mmHg. The effect of the crude water extract of E.hirta, acetylcholine and choline were investigated in the presence of pirenzepine - an M_1 subtype antagonist. The M_1 receptor is found in the cerebral cortexand smooth muscles. Identical doses of water extract, acetylcholine and choline that gave maximal fall in blood pressure (from 3.3.3A) were repeated in the absence and presence of pirenzepine (5 mg/kg). Water extract produced a maximum fall in MABP of 100% at a dose of 1mg/kg. Acetylcholine and choline produced maximum fall in MABP of 100% at 0.05 μg/kg and 0.5 mg/kg respectively.

Pretreatment of the cat with pirenzepine (5 mg/kg) reduced the effects of the leaf extract, acetylcholine and choline on MABP (Fig 3.3.3.9b). Pirenzepine reduced the maximum fall in MABP from 100% to 29% for water extract; for acetylcholine from 100% to 67% and for choline from 100% to 78%, respectively. The percentage blockade in the presence of pirenzepine was 71% for E.hirta water extract, 22% for acetylcholine and 33% for choline.

D **Effect of E.hirta on M_2 muscarinic receptor subtype using the isolated guinea pig atrium**

The arterial blood pressure depends on the cardiac output and the totalperipheral resistance. Therefore changes in the blood pressure (BP) can be brought about by alteration in cardiac output (CO), total peripheral resistance (TPR) or both. Other factors that affect the arterial BP are elasticity of the arteries, blood volume, viscosity of the blood and force of contraction (Tarverner, 1975). The specific muscarinic receptor in the heart is M_2 subtype. The effects of water extract, acetylcholine and choline on the force of contraction of the myocardium were evaluated based on the % relaxations produced on the isolated guinea pig atrium.

The crude water extract of E.hirta, acetylcholine and choline produced dose-dependent relaxations on the isolated atrium. The EC_{50} for acetylcholine was $0.125 \pm 1.04 \times 10^{-3}$ μg/ml, for choline $0.05 \pm 1.1 \times 10^{-3}$ mg/ml and water extract was 1.85 ± 0.86 mg/ml (Table 3.3.3.4). The water extract was not as potent as choline which was in turn less potent than acetylcholine. In the presence of methoctramine (0.6 μM) - which is a specific M_2 antagonist, there was partial blockade of the effects of acetylcholine (0.5 μg/ml) - 33.4%, choline (0.25 mg/ml) - 38.1% and water extract (2.5 mg/ml) which showed the most blockade 87.9% (Table 3.3.3.5 Fig. 3.3.3.8 and Fig. 3.3.3.9c).

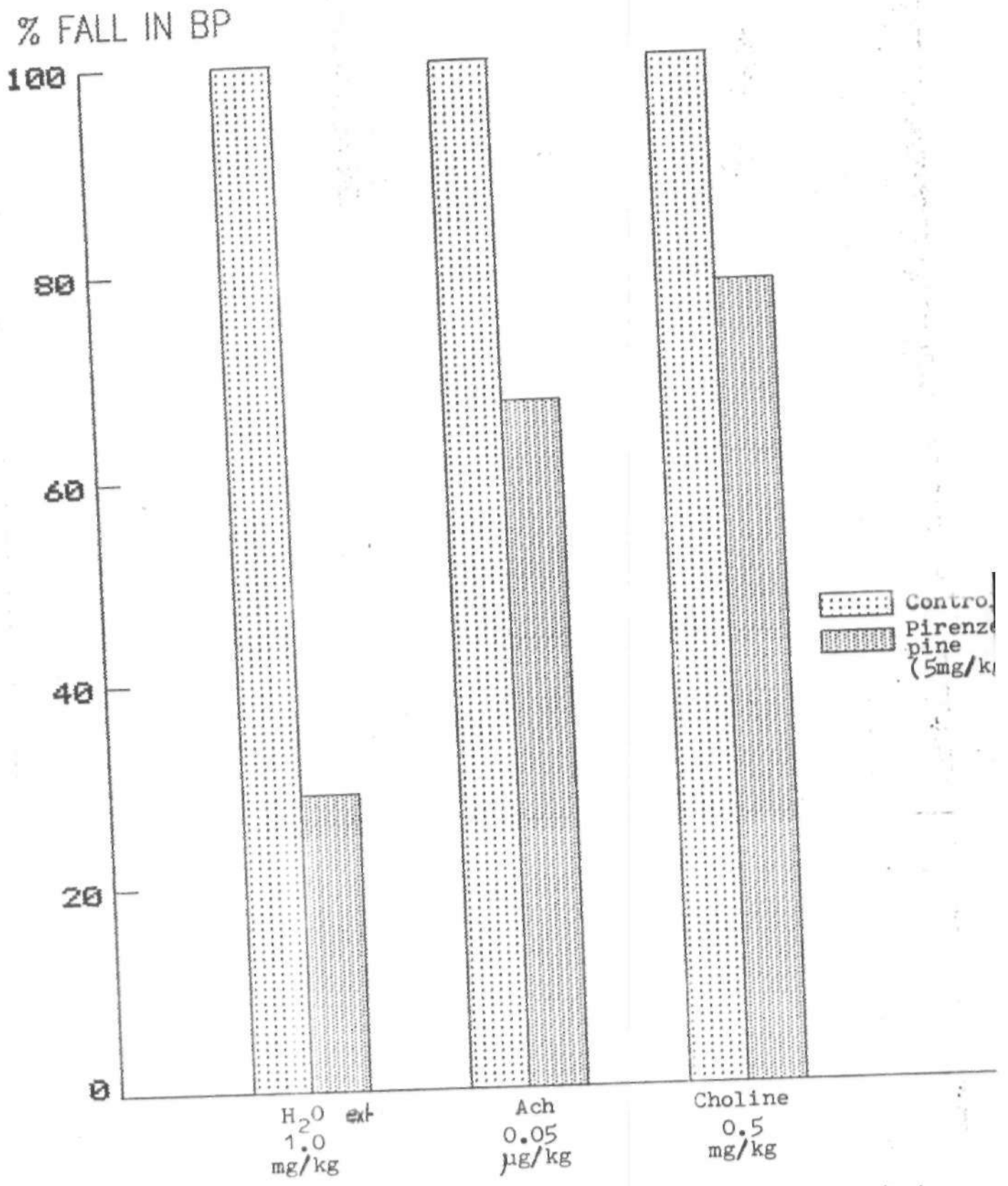


Fig. 3.3.3.9b. Dose related effect of pirenzepine (5 mg/kg) on the depressor action of E. hirta water extract, acetylcholine and choline on cat B.P.

Table 3.3.3.4: The relaxant effect of acetylcholine, choline and water extract on the isolated guinea pig atrium

	Concentration	Average % relaxation (cm)
Ach	0.025 μg/ml	14.3 ± 3.13
	0.05 μg/ml	28.6 ± 1.71
	0.125 μg/ml	50.0 ± 0.63
	0.25 μg/ml	85.7 ± 0.79
	0.5 μg/ml	93.8 ± 0.11
Choline	0.025 mg/ml	33.3 ± 1.37
	0.05 mg/ml	50.0 ± 1.46
	0.25 mg/ml	90.9 ± 0.91
H_2O extract	0.25 mg/ml	8.3 ± 1.01
	0.50 mg/ml	9.1 ± 2.17
	1.00 mg/ml	25.0 ± 2.63
	1.50 mg/ml	44.4 ± 3.84
	2.50 mg/ml	63.6 ± 3.16

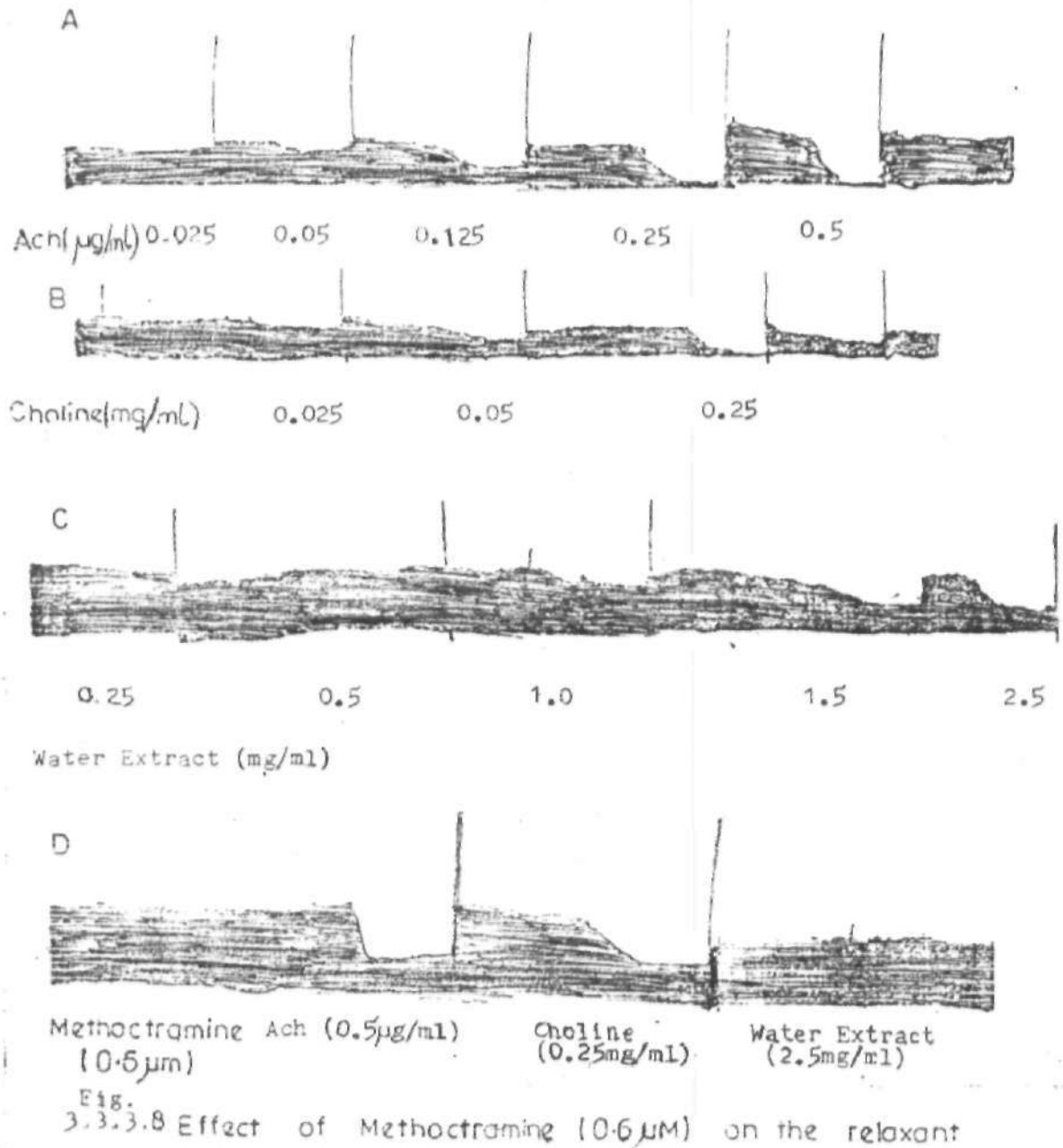


Fig. 3.3.3.8 Effect of Methoctramine (0·6 μM) on the relaxant effects of Ach, choline and Water extract of E. hirta leaves on guinea pig isolated atrium.

Table 3.3.3.5: The effects of acetylcholine, choline and water extracts on isolated guinea pig atrium in the absence and presence of methoctramine (0.6 μm)

	Average % relaxations		% Blockade
	Before methotramine	After methotramine	
Ach (0.5μg/ml)	93.8 ± 0.11	62.5 ± 3.31	33.4
Choline (0.25mg/ml)	90.9 ± 0.91	56.3 ± 1.79	38.1
H_20 extract (2.5mg/ml)	63.6 ± 3.16	7.7 ± 2.66	87.9

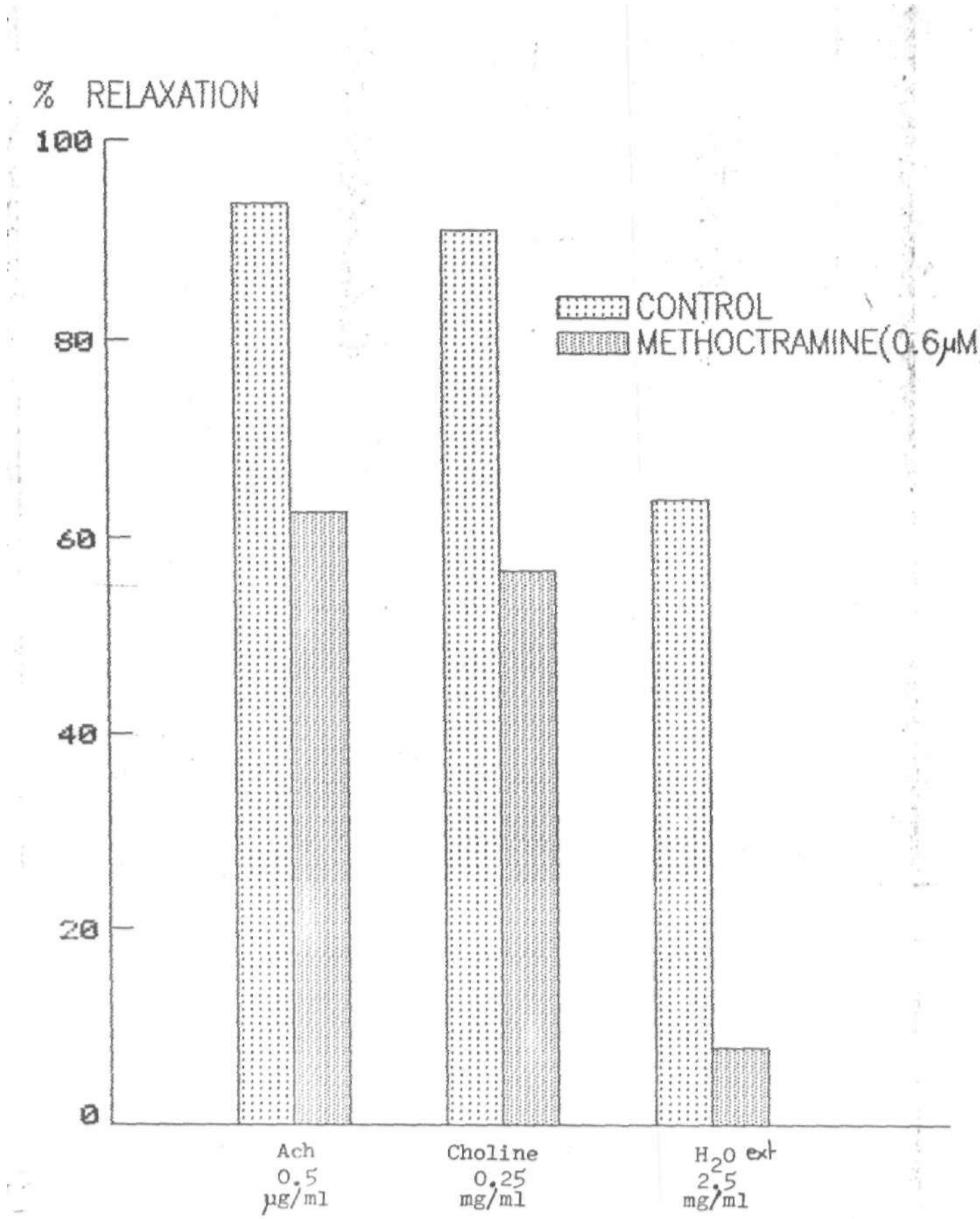


Fig. 3.3.3.9c. Dose related effect of Methoctramine (0.6 μM) on the relaxant action of acetylcholine, choline and water extract of E. hirta on isolated guinea-pig atrium.

3.3.4 **Effect of E. hirta leaf extracts on urine output and urinary electrolytes**

The results of screening the water and ethanol extracts of the leaves of E.hirta for diuresis are shown in Table 3.3.4.1. The water extract showed little change in urine output (U.O) at the two doses employed (at 50 mg/kg, UO was 3.9ml; at 100mg/kg UO was 4.08ml after the 24h period.). However, for 100 mg/kg, urine output was significant at 6h, when compared to the control; whilst for 50mg/kg urine output was significant at 12h and this continued through the 24h experimental period ($p < 0.05$). Diuresis was induced after 1h with the higher dose (100 mg/kg) of water extract; this activity was minimal when compared to that obtained at the lower dose (50mg/kg) which induced diuresis after 3h (Fig 3.3.4.1). The diuretic effect of ethanol extract was significant at the lower dose (50 mg/kg) from 6h (UO was 13.9ml) ($p < 0.05$), however for 100 mg/kg it was only significant at 24h which shows little or no diuresis. Diuresis by the ethanol extract was induced at both doses after the first 3h (Fig. 3.3.4.2). Urine output increased gradually till 12h, a sudden rise occured between 12 and 24h and this is more prominent with the lower dose (50 mg/kg).

The control (saline) showed only slight increase in urine output within the 24 experimental hours (Table 3.3.4.1). Frusemide (5 mg/kg) induced a brisk and significant diuresis within 30 minutes of administration. This reached a maximum in 3h and stayed constant until about 12h, then increased gradually till 24h. (Fig. 3.3.4.3). Acetazolamide (5 mg/kg) induced diuresis within 30 min which gradually increased within the 24h experimental period (Fig. 3.3.4.4). However, diuresis was only statistically significant after 1h. Water extract (50 and 100mg/kg) increased the pH of the urine from 8.8 to 10 $\pm$ 1.46, and 8.8 to 9.0 $\pm$ 1.4 respectively. The ethanol extracts increased urine pH from 8.8 to 10.3 $\pm$ 0.49. Acetazolamide increased the urine pH from 8.8 to 10.1, thus making the urine in each case more alkaline. However frusemide (5mg/kg) had no significant effect on the urinary pH.

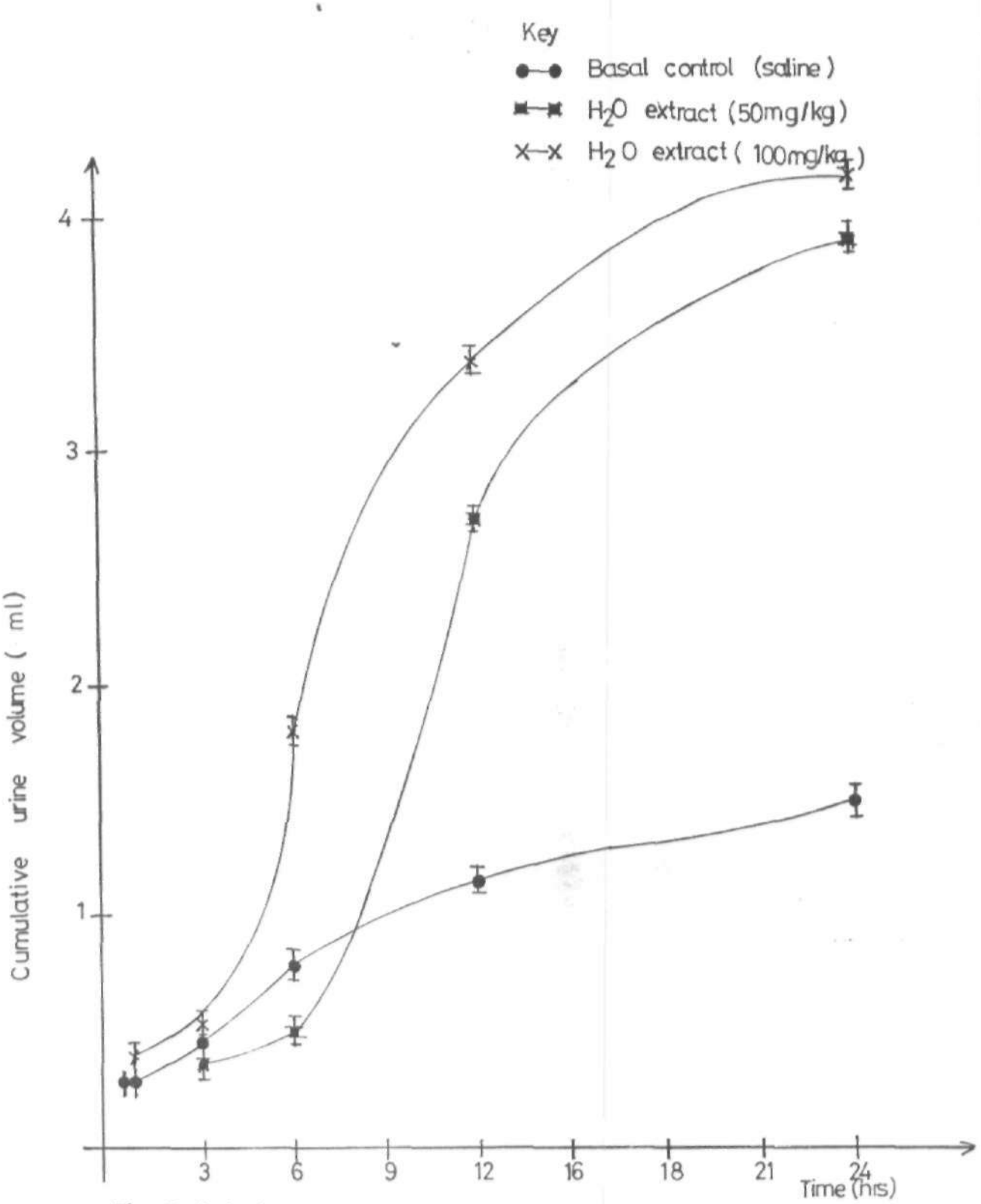


Fig 3·3·4·1

Diuretic effect of E. hirta leaf H_2O extract in rats
Each vertical bar is ± S·E·M (n = 4)

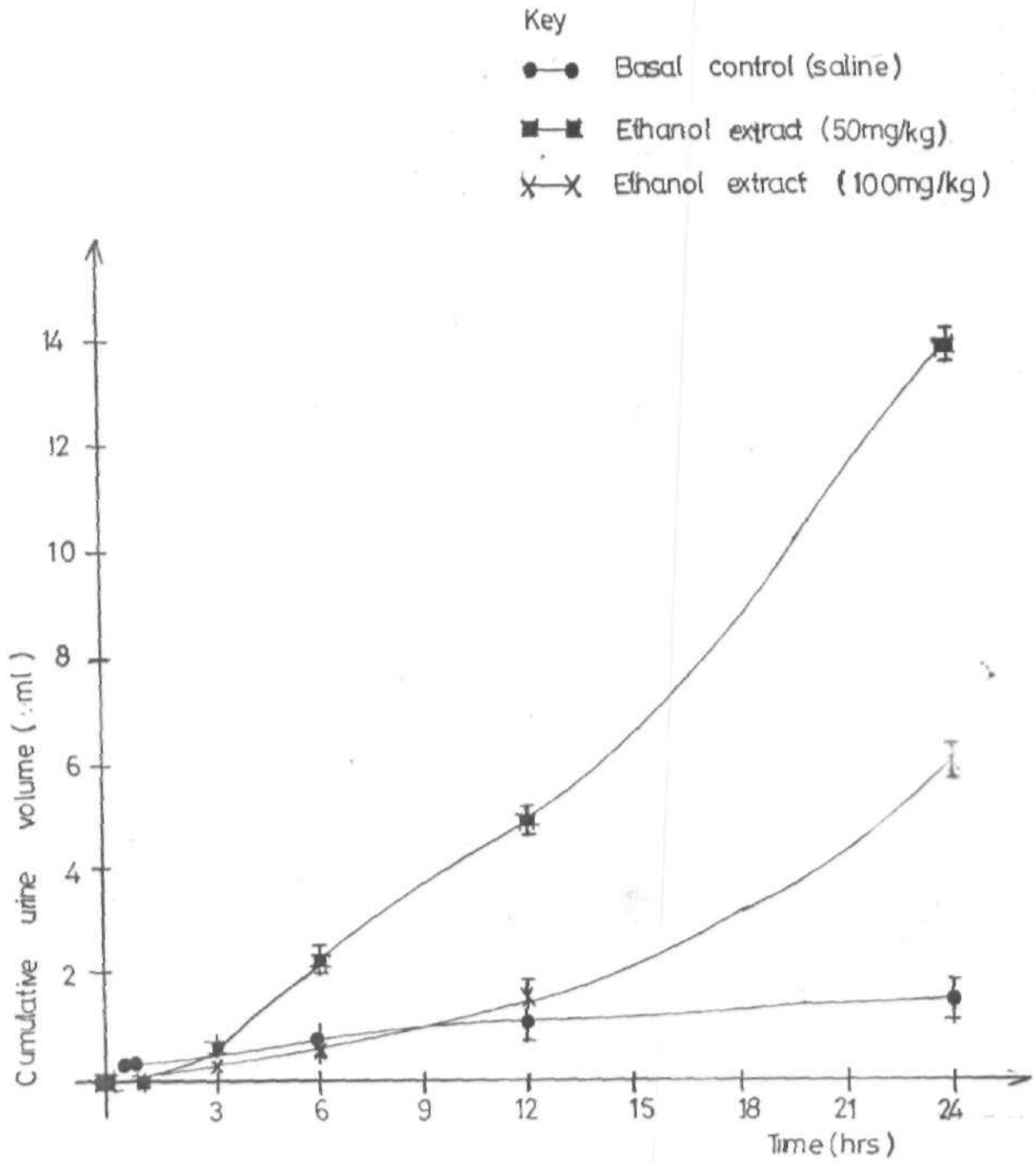


Fig 3·3·4·2

Diuretic effect of E. hirta leaf, ethanol extract in rats
Each vertical bar is ± S·E·M (n = 4)

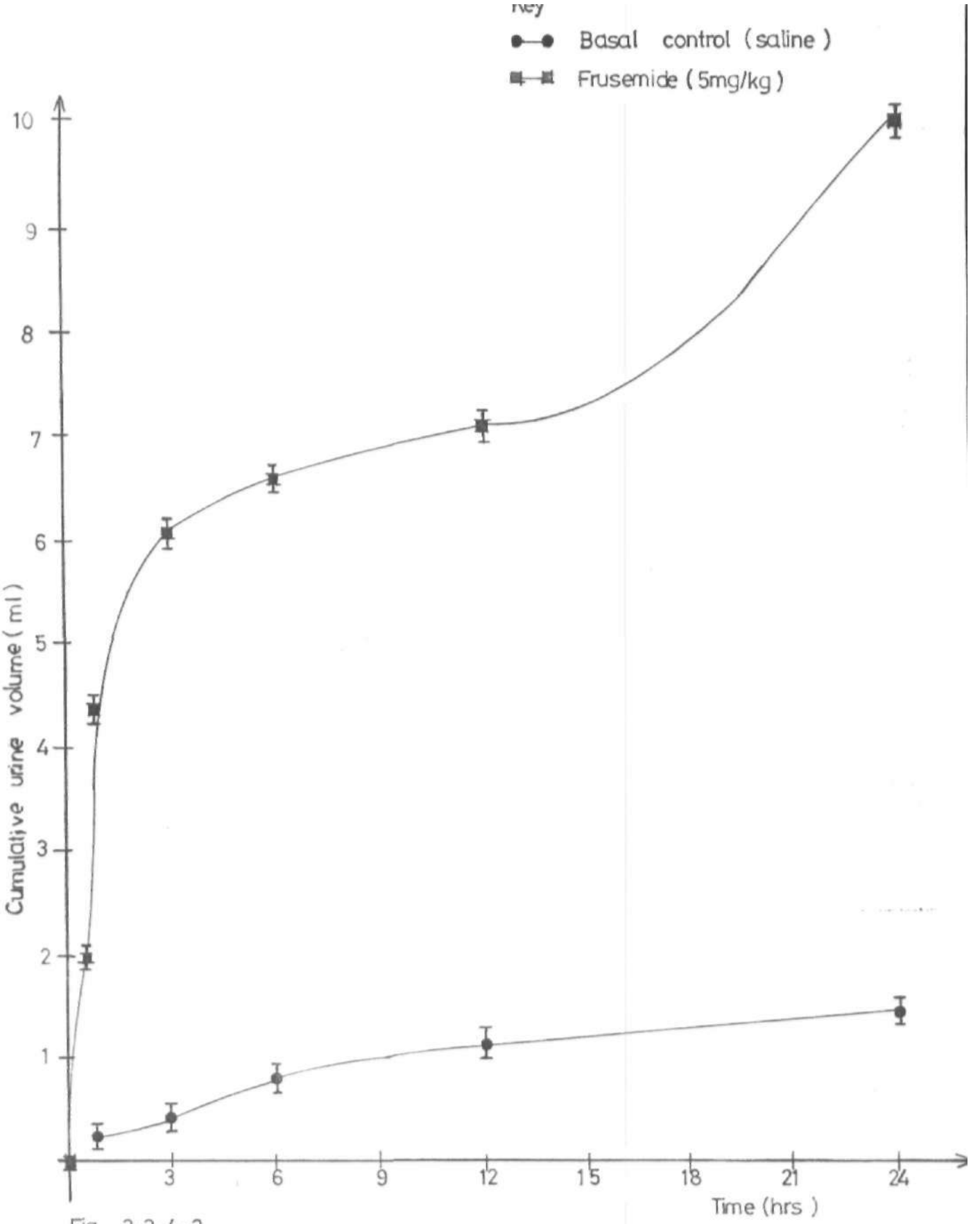


Fig 3·3·4·3

Diuretic effect of Frusemide (5mg/kg) in rats
Each vertical bar is ± SEM (n=4)

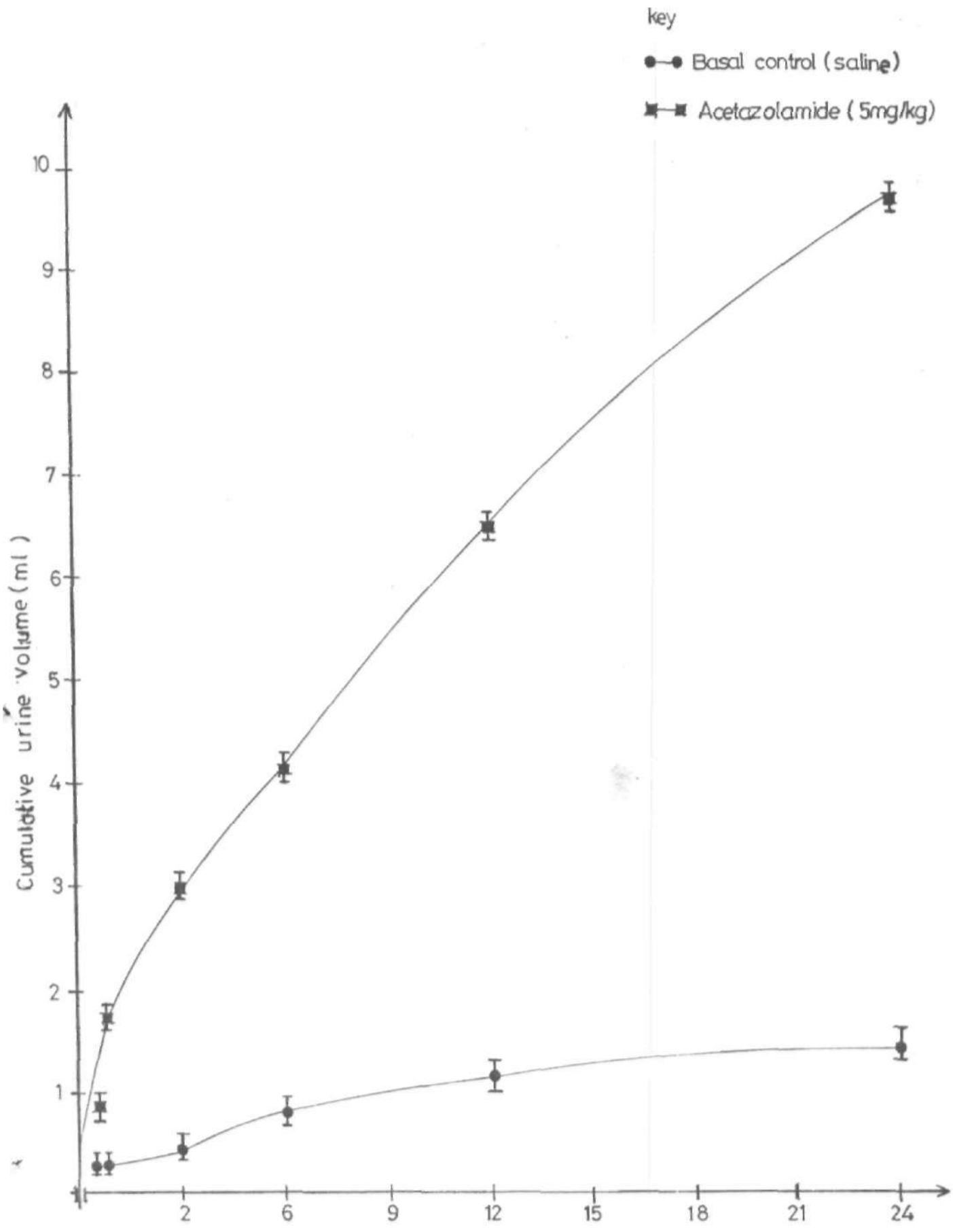


Fig 3·3·4·4
Diuretic effect of Acetazolamide (5mg/kg) in rats
Each vertical bar is± SEM (n=4)

Table 3.3.4.1: Diuretic response to water and ethanol extracts of E.hirta, frusemide and acetazolamide in rats

Time (h)		Urinary Output (ml)					
	0	0.5	1.0	3.0	6.0	12.0	24.0
Control (Saline)	0	0.27±0.16	0.3±0.05	0.43±0.08	0.8±0.16	1.17±0.28	1.6±0.33
H_20 extract (50mg/kg)	0	0	0	0.37±0.12	0.5±0.24	2.7±0.2*	3.9±0.74*
H_20 extract (100mg/kg)	0	0	0.4±0.08	0.5±0.12	1.8±0.4*	3.2±0.12*	4.08±0.3*
EtOH extract (50mg/kg)	0	0	0	0.6±0.48	2.2±0.21	4.9±0.4	13.9±3.8
EtOH extract (100mg/kg)	0	0	0	0.3±0.16	0.6±0.08	1.5±0.47	6.0±2.3**
Frusemide (5mg/kg)	0	2.0±0.07*	4.4±1.3*	6.1±0.31*	6.6±0.53*	7.1±0.18*	10.0±0.05*
Acetazolamide (5mg/kg)	0	0.85±0.43	1.73±0.54*	2.98±0.98*	4.13±1.12*	6.45±1.76*	9.7±2.28*

* reprosents $P<0.05$ student compared to saline conrols; n = 4.

The corresponding electrolyte changes induced by the various agents are recorded in Table 3.3.4.2. Water extract induced a significant increase in urinary Na^+, K^+ and HCO_3^- loss. Ethanol extract (100mg/kg) caused a significant decrease in K^+ loss whereas the water extract increased its excretion. The HCO_3^- output for both extracts were tremendous (with that of the lower doses being higher). Acetazolamide enhanced Na^+, K^+ and HCO_3^- loss significantly. The pronounced HCO_3^- excretion is expected, as it is a carbonic acid anhydrase inhibitor. Frusemide caused the expected rise in Na^+ and Cl^- excretion; but had relatively no effect on K^+ and HCO_3^- loss.

Table 3.3.4.2: Electrolyte changes in the urine of rats after 24h of "drug" administration (n=4)

	Electrolyte concentration ± S.E.M. (millimol/litre)				
	Na^+	K^+	Cl^-	HCO_3^-	pH
Control (Saline)	52.7±7.76	90±7.33	12.7±3.91	123±8.09	8.8
H_2O extract (50mg/kg)	104±4.24*	291.3±3.61*	25.3±2.24*	430±3.63*	10.0±1.46
H_2O extract (100mg/kg)	74±3.29*	180±3.33*	20.0±2.23	320±4.62*	9.0±1.4
EtOH extract (50mg/kg)	65±10.31	71.5±4.99	13.5±2.77	455±9.12*	10.3±0.49*
EtOH extract (100mg/kg)	60±5.71	39.5±3.60*	11.0±2.0	270±10.63*	10.0*
Frusemide (5 mg/kg)	106±0.38*	88±2.81	40±4.90*	120±1.49	8.9±0.81
Acetazolamide (5mg/kg)	106±7.80*	355±10.2*	18±2.53	530±10.54*	10.1±10.76

* represents $P<0.05$ student t-test sompared to saline controls; n=4.

3.3.5 Acute toxicity of E.hirta leaf extracts in mice

The signs and symptoms observed are presented in Table 3.3.5.1 and the severity of the symptom increased with an increase in dose of the extracts. Spontaneous micturition and defeacation appeared immediately followed by pallor and prostration and respiratory distress which was noticed in the first 3h of the administration of the extract. Sedation and coma always preceded (30-60 min) death.

Both water and ethanol extracts produced dose-related mortality of the mice (Table 3.3.5.2). The lowest dose that caused death was 0.4 g/kg for water extract and 1.0 g/kg for ethanol extract. The average time of death decreased with an increase in the dose of either water or ethanol extract. At higher doses (2.0 and 5.0 g/kg) the ethanol extract seems to kill the mice faster (0.45 to 2h) than the water extract (8 to 12h).

A **Calculation of LD_{50} values**

i. <u>Arithmetic Method of Karber</u>

In this method, the difference between two consecutive doses and the mean of the number of animals that died at two dose levels (mean dead) were calculated (Table 3.3.5.3a & b). The sum of the products of the mean dead and the dose difference was divided by the number of animals in each group. The resulting quotient was subtracted from the dose that killed all the animals in order to obtain the LD_{50} values and these are 1.26 g/kg and 2.0 g/kg for water and ethanol extracts, respectively.

Table 3.3.5.1: Signs and symptoms of acute toxicity of extracts of E.hirta in mice over a period of 24h

Signs and symptoms	Approximate time of appearance of sign/symptom after injection of toxic dose of E.hirta extracts (h)
1. Spontaneous micturition and defaecation	1/2
2. Pallor and prostration	1/2
3. Respiratory distress	1
4. Sedation	2
5. Coma	21/2 - 6
6. Death	3 - 15

Table 3.3.5.2a: The toxic effect of water extract of the leaves of E. hirta on the mortality of mice

Group	Dose (g/kg ip)	Ratio of Dead/Total	Average time of death (h)
1	0.10	0/5	-
2	0.20	0/5	-
3	0.40	2/5	15.50
4	1.00	3/5	13.01
5	2.00	4/5	12.08
6	5.00	5/5	8.93

Table 3.3.5.2b: The toxic extract effect of ethanol extract of leaves of E. hirta on the mortality of mice.

Group	Dose (g/kg ip)	Ratio of Dead/Total	Average time of death (h)
1	0.10	0/5	-
2	0.20	0/5	-
3	0.50	0/5	-
4	1.00	2/5	15.72
5	2.00	3/5	2.01
6	5.00	5/5	0.45

Table 3.3.5.3a: LD_{50} value calculated for water extract (n = 5)

Group	Dose (g/kg)	Dose difference (g/kg)	Dead	Mean Dead	Product of difference and Mean Dead
1	0.10	0.10	0	0.0	0.00
2	0.20	0.20	0	1.0	0.20
3	0.40	0.60	2	2.5	1.50
4	1.00	1.00	3	3.5	3.50
5	2.00	3.00	4	4.5	12.00
6	5.00		5		=18.70

* LD_{50} value = $5.00 - \frac{18.7}{5} = 1.26$ g/kg

Table 3.3.5.3b: LD_{50} value calculated for ethanol extract (n = 5)

Group	Dose (g/kg)	Dose difference (g/kg)	Dead	Mean Dead	Product of difference and Mean Dead
1	0.10	0.10	0	0.0	0.00
2	0.20	0.20	0	0.0	0.00
3	0.50	0.50	0	1.0	0.50
4	1.00	1.00	2	2.5	2.50
5	2.00	3.00	3	4.0	12.00
6	5.00		5		=15.00

* LD_{50} value = $5.00 - \frac{15.0}{5} = 2.00$ g/kg

Arithmetic Method of Reed and Muench

This method employs cumulative values. It is assumed that an animal killed by a certain dose would have been killed by a larger dose, and that a surviving animal would have survived a smaller dose. The cumulative dead are recorded by adding successive entries in the third column of Table 3.3.5.4a and b upwards. The cumulative survivors are recorded by adding successive entries of the fourth column downwards. Group 1 is neglected for both extracts (in addition Group 2 is also neglected for the ethanol extract) since doses smaller than the greatest, allowing all animals to survive and doses larger than the least causing all animals to die have no significance. The percent survival for both doses adjacent to the LD_{50} is computed and multiplied by the logarithm of the proportionate dose increment. This product is added to the logarithm of the smaller adjacent dose to form the logarithm of the LD_{50}. The LD_{50} is the antilog of this number.

iii. **Graphical method of Litchfield and Wilcoxon**

In fig. 3.3.5.1, the percent dead is plotted against the log dose of water and ethanol extracts. The LD_{50} values are determined from the graph and are 0.8 and 1.40 g/kg for water and ethanol extracts respectively. The mean LD_{50} values for water and ethanol extracts determined from the three independent methods are presented in Table 3.3.5.5.

Table 3.3.5.4a: LD_{50} value for water extract (n=5)

Group	Dose (g/kg)			Cumulative			%
		Dead	Survived	Dead	Survived	Total	Survival
1	0.10	0	5	14	-	-	-
2	0.20	0	5	14	5	19	26.3
3	0.40	2	3	14	8	22	36.4
4	1.00	3	2	12	10	22	45.5
5	2.00	4	1	9	11	20	55.0
6	5.00	5	0	5	11	16	-

a) Calculations

$$\frac{50 - 45.5}{55 - 45.5} = 0.474$$

$$\frac{2000}{1000} = 2$$

$0.474 \log 2 = 0.1426$

$\log 1000 = 3.0000$

$\log LD_{50} = 3.1426$

Antilog 3.1426 = 1389mg/kg

LD_{50} = 1.389g/kg

b) $\frac{50 - 36.4}{55 - 36.4} = \frac{13.6}{18.6} = 0.73$ LD_{50} = 1.294 g/kg

c) $\frac{50 - 26.3}{55 - 26.3} = \frac{23.7}{28.7} = 0.83$ LD_{50} = 1.336 g/kg

Average LD_{50} value (LD_{50}) = 1.34 ± 0.03

Table 3.3.3.4b: LD_{50} value for Ethanol extract (n=5)

Group	Dose (g/kg)			Cumulative			%
		Dead	Survived	Dead	Survived	Total	Survival
1	0.10	0	5	10	-	-	-
2	0.20	0	5	10	-	-	-
3	0.50	0	5	10	5	15	33.3
4	1.00	2	3	10	8	18	44.4
5	2.00	3	2	8	10	18	55.5
6	5.00	5	0	5	10	-	-

a) Calculations

$$\frac{50 - 33.3}{55 - 33.3} = 0.7523$$

$$\frac{2000}{500} = 4$$

0.7523 log 4 = 0.4529
log 500 = 2.6989
log LD_{50} = 3.1518

Antilog 3.1518 = 1419mg/kg
LD_{50} = 1.419g/kg

b) $\frac{50 - 44.4}{55.5 - 44.4} = 0.5045$

$$\frac{2000}{1000} = 2$$

0.5045 log 2 = 0.1518
log 1000 = 3.0000
log LD_{50} = 3.1518

Antilog 3.1518 = 1419mg/kg
LD_{50} = 1.419g/kg

Average LD_{50} value (LD_{50}) = 1.419 g/kg

Table 3.3.5.5: LD_{50} values for water and ethanol extracts of the leaves of E.hirta

	LD_{50} values (g/kg)			
Extract	Arithmetic Method of Karber	Arithmetic Method of Reed and Muench	Graphical method of Litchfield & Wilconxon	Mean ± S.E.M
Water	1.26	1.34	0.80	1.13 ± 0.21
Ethanol	2.00	1.42	1.40	1.61 ± 0.24

Table 3.3.5.6: The effects of various receptor antagonist and enzyme inhibitors on E.hirta leaf extract induced mortality in mice.

Receptor Enzyme	Antagonist or Inhibitor	Dose (g/kg)	Ratio of Death Total mice used	Average time of death (h)
Control	-	25ml	10/10	8.98±0.69
Muscarinic	Atropine	5.0μg 10.0"	4/10 7/10	2.15±0.90 0.03±0.79
Opioid	Naloxone	0.5mg	9/10	4.50±0.82
Cyclooxygenose	Indomathacin	0.5" 1.0"	10/10 10/10	2.98±0.49 8.03±0.37
Kallikrein	Aprotinin	10,000u 20,000u	10/10 10/10	8.14±0.91 8.30±0.73
PAP	WEB 2086	0.5mg 1.0"	8/10 7/10	2.16±0.43 1.62±0.68
5-lipoxygenase	NDGA	0.5" 2.0"	0/10 8/10	- 6.87±2.20
Phospholipase	Hydrocortisone	0.1" 0.2"	2/10 2/10	8.30±1.03 8.58±1.08

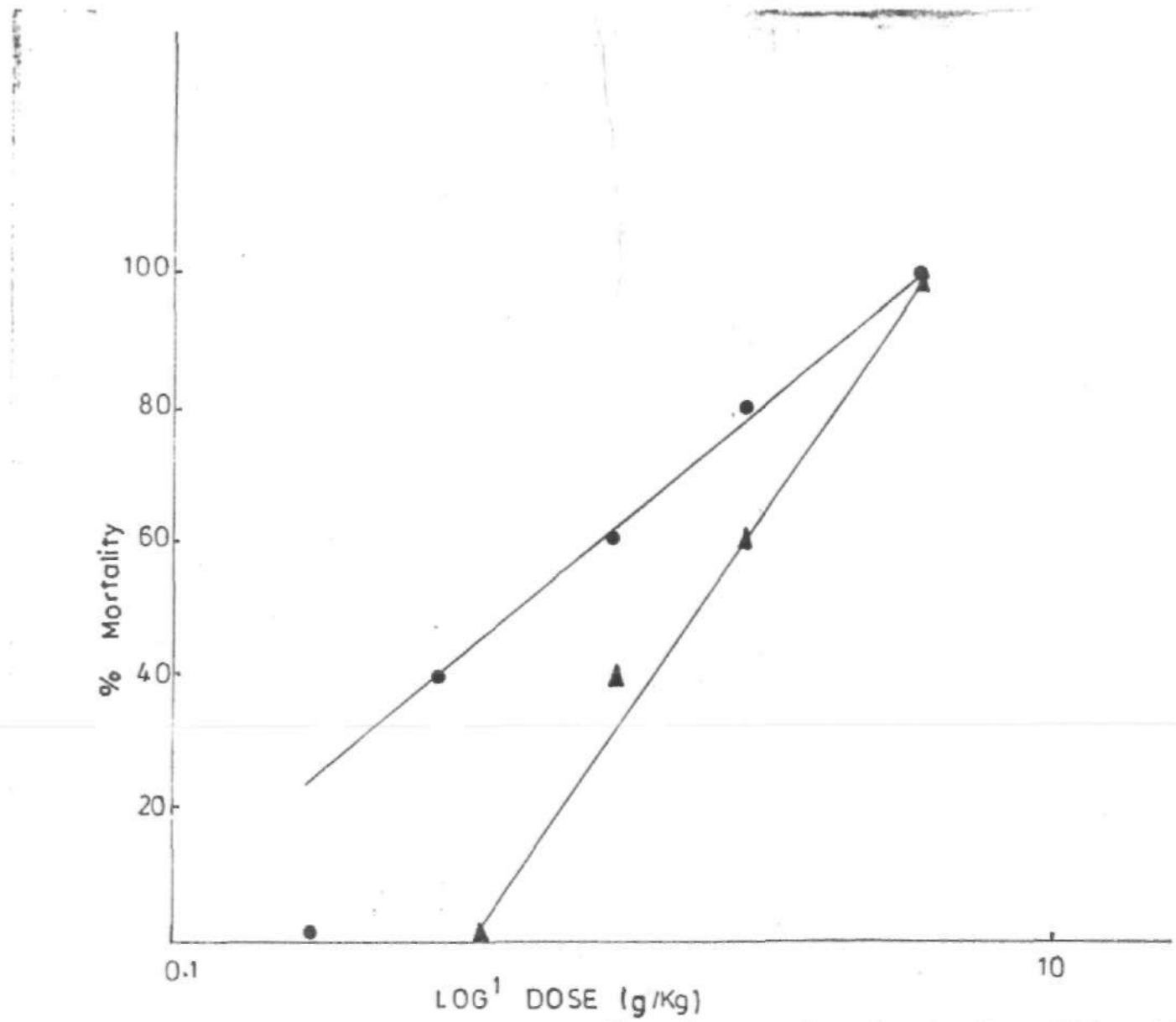


FIG. 3351 Log dose — percent mortality of water (●—●) and ethanol (▲—▲) extracts of the leaves of E. hirta using Mice (n = 5).

B **Mechanism of death induced by leaf extract of E.hirta**

From the signs and symptoms preceeding death, it is clear that the mice died of respiratory distress or failure. Coma also preceeded death. In these studies, drugs that block the generation or receptors for bronchoconstricting substances were used to elucidate the mechanism of death. Since the crude extract of this plant was reported to contain choline (a partial muscarinic agonist) - a muscarinic receptor antagonist was also employed in some of the studies.

Data presented in Table 3.3.5.6 indicates that muscarinic agonist(s), leukotriences, PAF and other compounds produced via phospholipase pathways maybe involved in the death produced by E.hirta. Atropine (5 μg/kg) protected the mice by 60% and this was reduced to 30% when a higher dose (10 μg/kg) was used. NDGA (0.5 mg) offered 100% protection to the animals against the water extract-induced death. This was reduced by only 20% when the dose was increased to 2mg/kg. WEB 2086 (a PAF-receptor antagonist) reduced the mortality of the mice by 20% and 30% when 0.5mg/kg and 1.0mg/kg respectively, when it was administered 30 min prior to E. hirta injection. Hydrocortisone protected the mice from the toxicity of the extract, by 80% when 0.1 and 0.2 mg/kg were used 60 min before the administration of the plant. Naloxone (0.5 mg/kg) protected the mice by 10% against the water extract- induced death. Aprotinin (10,000 and 20,000 u/kg) and Indomethacin (0.5 and 1.0mg/kg) offered no protection. For WEB 2086 (1.0 mg/kg) death occurred almost immediately within 1.62h.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

Plants have provided a major source of medicines since the earliest times known to man. These natural drug products have yielded a number of compounds useful as investigative tools in pharmacological studies - some of which are of immense therapeutic values. Some have provided templates for the synthesis of more potent or clinically useful analogues. The folkloric approach to the use of certain plants for medicinal purposes has led to the identification, isolation and characterization of some compounds. Some of which include digitoxin from Digitalis purpurea, reserpine from Rauwolfia serpentine, morphine from Opium species (Phillipson et al, 1989). In this project, an attempt has been made to investigate the pharmacological rationale for the use of the leaves of Euphorbia hirta in some traditional remedies. Other unreported pharmacological properties (and mechanism of action) of the leaves of E.hirta were also investigated.

4.1 IN VITRO STUDIES

4.1.1 Effect of water and ethanol extracts of the leaves of E.hirta on rabbit jejunum

An infusion of the plant E.hirta has been used extensively in the treatment of diarrhoea, dysentery and colic (Watt et al, 1962; Sussman, 1980, Oliver, 1983, Sofowora, 1984). The causes of diarrhoea are legion, but the overall alterations of intestinal function are similar. The intestine ceases, at least along a part of its length to be an organ of net absorption of water and electrolytes, and to the extent that fluid produced exceeds the absorptive capacity of the remaining intestine, water thus passes into the stool (Binder, 1989). One of the aims in the treatment of diarrhoea is decreasing intestinal motility (thereby

favouring absorption). This is also important in the treatment of colic (Brunton, 1990). The activity of the isolated rabbit jejunum is controlled to a considerable extent by local mechanisms which retain much of their function in the isolated preparation; thus substances which have action on the intestine in vitro may act directly on the muscle fibres or directly via the surviving autonomous plexuses (Crossland 1982). Parasympathetic agents contract the jejunum whereas the sympathetic agents have a predominantly inhibitory action on this tissue.

The water extract of E.hirta produced prominent relaxation at low concentration (0.025 mg/ml). However, the ethanol extract showed biphasic action with relaxations being more prominent than contractions. The activity in the ethanol extract resides mainly in fraction 28 following fractionation of this extract.

To investigate the mechanism of action involved in the water extract induced-relaxations of the rabbit jejunum, propranolol (a non-selective β-adrenoceptor antagonist), pirenzepine (a selective M_1 muscarinic receptor antagonist) and atropine (a non-selective muscarinic receptor antagonist) were used. Atropine and pirenzepine did not have any significant effect on the extracts. Pirenzepine possibly did not produce any antagonism because the main muscarinic receptors in the jejunum are of the M_3 subtype. Atropine was unsuitable because it even inhibited the spontaneous contraction of the tissue.

However, propranolol (1 μM) antagonized the relaxation produced by the water extract implying the possible involvement of the sympathetic system particularly β-adrenoceptor(s). These results obtained with ethanol and water extracts of the leaves of E.hirta are not in agreement with that reported by Hazelton and Hellerman (1954) who did not observe any effect on the rabbit gut when they used aqueous as well as alcoholic extracts of the same plant. However, this could be due to the presence of antagonistic components

which are commonly found in plant extracts or the insensivity of the portion of gut used (for the jejunum has been found to be the most sensitive). These are problems normally encountered in Ethnopharmacology (Malone, 1977).

The water and ethanol extracts are used in colic and diarrhoea, therefore one of the probable underlying mechanisms involved in the treatment of these disease states with E.hirta could be due to the relaxation of the intestinal smooth muscle. Relaxation of this muscle will decrease intestinal motility which relieves colic and further favours water absorption in diarrhoea. The plant has also been widely used in dysentery, therefore antimicrobial studies were carried out on the extracts. This study suggests that the extracts and fraction 28 might be bacteriostatic agents rather than bactericidal.

Presently, we are in the process of characterising the active principle in fraction 28.

4.1.2 Effect of extracts on platelets

The flowering and fruiting plant of E.hirta are used in treating asthma and respiratory tract inflammations (Oliver, 1959; Kokwaro, 1976). It is also used in the treatment of chronic bronchitis, coughs and other pulmonary disorders (Wong-Ting Fook, 1980). Asthma is now one of the leading causes of hospital admissions in the paediatric population (Reed, 1986; Rall, 1990). Despite increased availability of good anti-asthmatic medications, the rate of asthma-related death is actually rising (Bentor, 1986, Reed, 1986). Some of the mediators include histamine, eicasonoids (i.e. leukotrienes and prostanoids), adenosine, proteases and platelet activating factor (PAF) (Morley et al, 1984; Moore, 1985; Rall, 1990). In the last twelve years, PAF has been shown to induce pathophysiological conditions similar to those of asthma and a number of competitive (reversible and irreversible) PAF receptor antagonists have been isolated from plants and chemically synthesized. Since PAF and prostanoids have

been postulated to be putative mediators of asthma and other inflammatory diseases, the leaf extracts of E.hirta were used to antagonize their effects or biosynthesis. The human platelets rich plasma (PRP) was used.

The ethanol extract had no aggregatory effect on the human platelets. Low concentrations of water extract (10 - 100 μg/ml) did not produce platelet aggregation. However, at higher doses (0.2 - 1mg/ml) concentration-dependent aggregation was induced. The aggregation was an irreversible, single wave. Subthreshold concentrations of water extract and the various concentration of the ethanol extract, did not have any effect on PAF-induced platelet aggregation; which was blocked by the selective PAF antagonist - WEB 2086. This suggests that the extracts might not have any significant effect on PAF action during an asthmatic attack; infact, the aggregation produced with higher doses of water extract could even aggravate an asthmatic attack.

Subthreshold concentrations of water extract and the various concentration of ethanol extract had no significant effect on adrenaline-induced platelet aggregation, which was partially blocked by the cyclooxygenase inhibitor - indomethacin. Adrenaline produces biphasic and reversible platelet aggregation, the first wave being receptor mediated and the other due to the effect of released substances (eg. prostanoids) on the platelets (Hussaini, 1986).

To investigate the mechanism of water extract induced platelet aggregation, the concentration that produced maximum platelet aggregation was used in the presence of a number of standard drugs. Indomethacin produced a dose-related inhibition of water extract -induced platelet aggregation. This suggests involvement of prostanoids in the action of the E.hirta water extract. However, since the blockade was not complete, it cannot be said to be solely mediated through the arcachidonate cascade. The extract probably acts via the

phospholipase - cyclooxygenase enzyme system to release either thromboxanes or leukotriences or a combination of any of these two which are putative mediators of asthma. This could support the claim of some of the traditional practitioners in Northern Nigeria, that E.hirta actually causes asthma rather than treat it. Also the mechanism underlying its toxicity supports this fact (Hussaini, personal communication).

Nifedipine, a calcium channel blocker with selective vasodilation ability, also reduced the water extract-induced platelet aggregation considerably. The aggregation caused by the extract is most probably calcium ion dependent. Calcium is normally coupled to myosin light chain kinase which undergoes phosphorylation to produce contraction in smooth muscles. This contraction is blocked in the presence of a calcium channel blocker like nifedipine (Murad, 1986). Similar phenomenon also takes place in the platelets.

The proposed mechanisms have all seemed to contradict, the main use of E.hirta as the "asthma herb"; however, this is not conclusive to say that it does not treat asthma as only 3 out of the 6 or more mediators were investigated.

4.2 IN VIVO STUDIES

Effect of E. hirta leaf extracts on cat blood pressure and isolated guinea pig atrium

Dickshit (1934) reported that the toxic principle in E. hirta depressed the cardiovascular system with a resulting fall in blood pressure (BP). The alcoholic and aqueous extracts both depressed the blood pressure of the dog (Hazleton et al., 1951). None of these studies investigated the mechanism involved in the fall in BP caused by E.hirta. The water and ethanol extracts of E. hirta had depressor effects on the mean arterial blood pressure (MABP). The cholinergic agonists-acetylcholine and choline also had similar effects. Choline was tested because it has been reported to be one of the constituents of the

water extract (Oliver, 1983; Sofowora, 1984). The observed depressor effects was partially blocked by atropine (a non-selective muscarinic receptor antagonist). This may suggest that the extract most likely acts via muscarinic receptor(s).

A further study to ascertain the muscarinic receptor subtype(s) involved, revealed that the extract acts on both M_1 and M_2 muscarinic subtype - which is similar to the action of acetylcholine and choline. M_1 receptors are located at the autonomic ganglia of the central nervous system. The stimulation of this receptor leads to the release of both acetylcholine and noradrenaline, with the former's effect being more prominent as the parasympathetic nervous system predominates over sympathetic nervous system. The net result is a fall in blood pressure (Hammer et al, 1982. Brown, 1990). Pirenzepine (a M_1 receptor antagonist) causes more blockade on the depressor effect of the water extract than on the cholinergic agonists. This suggests a more significant activity of the extract on the M_1 receptor than that obtained with the cholinergic agonists. This blockade indicates that the water extract maybe producing hypotension via dilation of blood vessels with a consequent change in total peripheral resistance.

Since changes in blood pressure could arise from alterations in the cardiac output, as well as in total peripheral resistance, the effect of the extract was tested using an M_2 - muscarinic receptor subtype model - isolated guinea pig atria. The extracts reduced the amplitude of contraction of the atrium but its effect on the heart was less pronounced in comparison with that of acetylcholine and choline. The extract most probably acts via M_2 receptors which are located in the heart. Therefore, the decrease in blood pressure by the water extract is partly due to reduction in the myocardal contraction and subsequently reduction in cardiac output. Furthermore, methoctramine - an M_2 muscarinic receptor

subtype antagonist inhibited the atrial relaxations induced by the water extract; thus supporting the fact that the water extract acts via myocardial M_2 receptors.

This study has established the effects of E.hirta leaf extracts on blood pressure and the possible mechanism of the observed hypotension. This effect might be as a result of muscarinic receptor activation (specifically M_1 and M_2 subtypes) by the choline (a muscarinic agonist) in the water extract. This effect supports the pharmacological observations of Dickshit (1934) and Hazleton et al (1951). However, E.hirta is presently not used for treating hypotensive cases in traditional medicine.

4.2.2 Effect of E.hirta leaf extracts on Urine output and Urinary electrolytes

The Swahili and Sukumas use E.hirta as a diuretic remedy (Watt et al, 1962). Diuresis has two separate connotations: one refers to the increase in urine volume per se, the other to the net loss of solute (ie. electrolytes) and water (i.e. saluretic effect) (De Stevens, 1963; Irwin, 1990). These two processes are involved in the suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into the blood stream, and as a consequence, promote the formation of urine (De-Stevens, 1963; Milton et al, 1970).

Diuresis was induced in rats treated with the water and ethanol extracts of E.hirta. The mechanism of action by which diuresis was induced by the extracts was also investigated by comparing with standard reference drugs namely frusemide (a high- ceiling loop diuretic) and acetazolamide (a carbonic anhydrase inhibitor). Frusemide (Lasix$^{(R)}$) induced a brisk and significant diuresis within 30 mins of administration. It increased sodium ion (Na^+) and chloride ion (Cl^-) excretion with relatively no effect on potassium ion (K^+) and bicarbonate ion ($HCO3^-$). It acts by inhibiting electrolyte reabsorption in the thick, ascending limb of the

Loop of Henle. Greger and Wangermann (1987) found from micropuncture experiments that high ceiling diuretics enhanced Na^+ and Cl^- excretion; and microperfusion experiments revealed that there was complete inhibition in vitro at luminal concentration of drugs in the range expected to occur in vivo. It was also observed that the high ceiling diuretic enhanced excretion of magnesium ion (Mg^{+2}) and calcium ion (Ca^{+2}) to an extent approximately equal to the increase in Na^+ excretion and they also cause a greater depletion of K^+.

Acetazolamide (Diamox$^{(R)}$) an osmotic diuretic induced diuresis within 30 min of administration. It induced a significant increase in urinary Na^+, K^+ and HCO_3^- ions. The HCO_3^- excretion was pronounced and this is expected as it is a carbonic anhydrase inhibitor. Physiological effects following acetazolamide administration, however became apparent only when about 99% of the enzyme activity is inhibited in the kidney (Irwin, 1990). Preisig et al (1987), further observed that acetazolamide increased the volume of the now alkaline urine and there was a slight urinary increase in HCO_3^-, accompanied by increases in Na^+ and K^+ but a decrease in Cl^-.

The water extract showed little change in urine output at the 2 doses employed but was statistically significant at 6h (for 100 mg/kg) and 12h (for 50 mg/kg). The diuretic effect of the ethanol extract was significant at 6h (for 50 mg/kg) and at 24h (for 100 mg/kg). From the earlier definition, it can be seen that there was great diuretic effect at the lower doses for both extracts; however this will not be complete if the net electrolyte loss is not considered alongside with the urine output. Both doses of the water extracts induced a significant increase in urinary Na^+ and K^+ loss whereas the ethanol extract (100 mg/kg) caused a significant decrease in K^+ loss. This suggests that an increase in the dose of the water extract can lead to hypokalemic alkalosis and this is in line with the pH of the urine, but the lower dose can lead to hyponatremia. However, the effect on K^+ is the reverse with

the ethanol extract which indicates that an increase in dose will lead to hyperkalemic acidosis and subsequent decrease in dose causing hypernatremia (Milton et al, 1970). The bicarbonate output for both extracts were tremendous, however the lower doses of both extracts gave more pronounced excretion. This is a characteristic of a carbonic anhydrase inhibitor.

The 24h cumulative urine output induced by the extracts and the drugs were statistically significant; but the urine output for frusemide was more than that of acetazolamide. However, the ethanol (50 mg/kg) gave the highest urinary output after 24h period. The leaf extracts of E.hirta were shown to exhibit two different mechanisms which maybe based on the different active components they contained. The water extracts behaved very similar to acetazolamide, for there was increased Na^+, K^+ and pronounced HCO_3^- - excretion. The pH was also alkaline. The water extract most probably acted via the distal convulated tubules. This is due to its effect on the Na^+ pump as well as its carbonic anhydrase inhibitory action.

Ethanol extract (100 mg/kg) caused a decrease in K^+ excretion (which will lead to hyperkalemia). The pH was also alkaline. However, though it caused a tremendous HCO_3^- excretion, it had relatively no effect on Na^+ or K^+. Therefore, the mechanism of action of the ethanol extract may mostly be due to carbonic anhydrase inhibition and definitely not on the loop of Henle, as it does not affect the Na+ excretion. It could probably act via the collecting duct or may be mostly due to the increased rate of urine formation rather than having any saluretic effect. However, it is still a diuretic agent.

The data presented in this thesis, suggest that E.hirta extracts (water and ethanol) contained diuretic agent(s) which mediated their effects by increasing rate of urine output as

well as electrolyte excretion. This supports the use of the herb by the Sukumas and the Swahili as a diuretic remedy.

4.2.3 Acute Toxicity of E.hirta leaf extracts in mice

In traditional medicine, the native "doctors" do not exactly quantify the amount of medication they instruct their patients to take. Infact, they do not know the amount and nature of the active principle(s) in the concoction they prescribe. In such a "blind" practice of prescription, the probability of administering toxic level of the medication is very high. A deliberate exploration of the toxic doses in humans is out of the question in the primary screening, therefore the initial qualitative and quantitative evaluation of the toxicity of E.hirta were carried out in mice. Eventhough, species differences between humans and mice are considerable yet this study will indicate the types of harmful effect to be expected and at what dose levels they may be manifested.

The extracts produced dose-dependent death with water extract causing death at lower doses than ethanol extract. This might indicate the presence of more toxic (or active) compounds in the water extract than in the ethanol extract. However, this may not be strictly true as the latter produced death in a short time interval at the highest dose (5 g/kg) than the water extract. The mean LD_{50} values are 1.13 ± 0.21 and 1.61 ± 0.24 for water and ethanol extracts, respectively. According to WHO (Dubois and Geiling, 1959) these values fall within the slightly toxic range (0.5 - 5.0 g/kg). In traditional medical practice, only a pinch of the powdered plant is administered orally with "kunu" or pap (Alhaji, Alhassan Likoro, personal communication). Therefore, the possibility of acute poisoning is very rare but the probability of accidental poisoning exists.

Several antagonists (or inhibitors) were employed in the investigation of the mechanism of death. Indomethacin and aprotinin offered no protection; thus death may not be due to the action of the extract on cycloxygenase enzyme in the arachidonate cascade or the kallikrein enzyme found in the plasma. There was little or no opiate receptor involvement, as shown by the action of naloxone. However, E.hirta may produce death via some muscarinic receptor involvement. This is due to the protection offered by atropine at both doses and further confirmed by the effect of the leaf extracts on blood pressure, where it is found to selectively act on M_1 and M_2 receptor subtypes.

WEB 2086 (a PAF receptor antagonist) offered some protection which may suggest the involvement of PAF. Since PAF has never been found in a plant, active principle(s) in the extracts maybe inducing the release of endogenous PAF in the mice from platelets, neutrophils or other cells. There is however a strong indication of arachidonate cascade dependence. This is due to the protection offered by inhibitors of 5- lipoxygenase (NDGA) and phospholipase (by hydrocortisone). NDGA offered 100% protection at 0.5 mg/kg, which reduced to 80% at 2 mg/kg. 5-lipoxygenease converts arachidonic acid to leukotrienes (LT) and other peptidase; some important leukotrienes are LTB_4, LTC_4 and LTD_4. LTC_4 and LTD_4 have been found to cause hypotension which may result from a decrease in intravascular volume and in cardiac contractility followed by a marked leukotriene-induced reduction in coronary bloodflow. They are also powerful bronchoconstrictors in many species including man (Feuerstein, 1984, Piper, 1984). This maybe the main cause of the respiratory distress observed in the animals after an hour of extract administrion. Hydrocortisone (a glucocorticord) offered 80% protection against death. Glucocorticoids inhibit phospholipase A_2 - the enzyme that converts phospholipids to arachidonic acid. This inhibition is however indirect through induction of the synthesis of a protein (lipocortin) that

inhibits the enzyme (Flower and Blackwell, 1979). Inhibition of phospholipase A_2 decreases the release of the precussor fatty acid and thus the synthesis of all metabolites derived thereof. This leads to accumulation of phospholipids.

Death due to E.hirta leaf extracts has thus been shown to be partly due to muscarinic receptor involvement but having a greater lean towards arachidonate cascade. Protection was offered by phospholipase and 5-lipoxygenase enzyme inhibitors. These enzymes catalyze different reactions in the cascade to give rise to numerous products some of which are potent vasoconstrictors, vasodilators bronchoconstrictors, platelet aggregating agents, etc, which probably play a very important role in the depression of cardiovascular system, with observed symptoms of respiratory distress, sedation and coma.

4.3 CONCLUSION

Based on this study, the water and ethanol extracts of the leaves of E.hirta have been shown to have relaxant effect on the rabbit jejunum (though ethanol extract produced slight contractions), this might be mediated via the β-adrenoceptor mechanism. This goes to support the widespread use of the plant for the treatment of diarrhoea and colic. Furthermore, the basteriostatic effect of the extract substantiates the use of this plant in dysentry.

E.hirta has been used widely as an "asthma herb" even though these claims have been disproved by some others. However from this study, investigation of the possible mediators of asthma supported the latter claim that E.hirta causes rather than treats asthma. The acute toxicity test also gave similar results. Traditionally E.hirta is not used as a hypotensive agent; however pharmacological screening of the leaf extracts showed hypotensive effect mediated via muscarinic receptors specifically M_1 and M_2 subtypes.

Investigation of the diuretic effect of the plant (employed by the Sukamas for this purpose) yielded significant diuresis with the water extract having a similar spectrum as that of acetazolamide. However, the mechanism of action of the ethanol extract was not clearly defined though there was substantial carbonic anhydrase inhibition. According to WHO standards, the LD_{50} values for both extracts fall within the slight toxic range (Dubois & Geiling, 1959). This may explain why there have not been cases of severe poisoning in literature.

4.4 LIMITATIONS AND SUGGESTIONS

In the extraction procedure, the cold percolation method was employed as heat destroys some of the active constituents. However, the water extract was dried in an oven at low temperature due to the large mass of water to be evaporated. Comparison should be made with an air-dried sample and an oven-dried sample by pharmacological screening. The mechanism of action of the relaxation produced by the water and ethanol extracts was investigated using atropine, pirenzepine and propranolol. This result shows the involvement of the sympathetic system namely β-adrenoceptors involved by using specific β-adrenoceptors like metoprolol (β_1) and α-adrenoceptor antagonists like phentolamine (non-specific), prazosin (α_1) and yohimbine (α_2) to examine the effect of the extracts on specific α-adrenoceptors.

The investigation of E.hirta's effect on platelets as a model for ascertaining its anti-asthmatic effect seemed to contradict this use. However, it is not conclusive to state that it cannot be used to treat asthma, as only three of the numerous mediators were investigated. The effect of E.hirta on other mediators like histamine, adenosine, proteases, etc. should be investigated. The untapped pharmacological effect-hypotension, should be further investigated using other cardiac preparations such as the Langendorff preparation; so as to

ascertain the exact point of action of E.hirta which maybe the SA or AV node, atrium or ventricle.

The diuresis produced by E.hirta maybe a result of a combination of known mechanisms of other diuretic agents. Therefore comparison should be made alongside with other diuretic agents from different classes such as mannitol, urea, chlorothiazide, benzthiazide, ethacrynic acid, bumetamide, spirinolactone, triamterene and others.

Other traditional uses should be investigated using appropriate pharmacological models. The use of the leaves to increase lactation and its aphrodisiac effect maybe linked to some effect on the pitiutary system, even though the extract had no appreciable effect on the isolated rat uterus. The healing effect of sores and wounds by the leaves of this plant should be closely investigated, as this might prove useful in the treatment of ulcers, especially due to its relaxant effect on the gut. However, phytochemical elucidation is necessary so as to isolate and further characterize the active principles based on pharmacological screening which are responsible for the stated traditional/folkloric uses.

There is no doubt that much can be gained from screening local remedies for pharmacological activity. The stated aim of WHO to provide "Health for all by the year, 2000" has further helped to focus attention on the systems of traditional medicine which are used extensively in all countries in the developing world (Bannerman et al, 1983). Therefore, it has become desirable that valid laboratory data be extrapolated to clinical situations, after further research to determine the mechanism of such effects and to characterize the active principle(s). Our goal of improved health for all can only be attained if we harness our natural resources in our development process.

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APPENDIX I

PREPARATION OF PHYSIOLOGICAL SALT SOLUTIONS

	Quantities for 10 litres :		
	KREBS	TYRODE	Mc-EWEMS
NaCl	69g	90g	76g
10% KCl	35ml	20ml	42ml
10% $MgSO_4 . 2H_2O$	20ml	-	-
10% $NaH_2PO_4 . 2H_2O$		5ml	14.3ml
10%KH_2PO_4	ml		
D. Glucose			
$NaHCO_3$			
$CaCl_2$ (molar)			
10% CaCl			
Sucros			45g
Aerating		Air	O_2 or Air