MYRICA SALICIFOLIA (MYRICACEAE): AN INVESTIGATION INTO THE PHARMACOLOGICAL BASIS OF ITS USE IN TRADITIONAL MEDICINE

By

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I hereby declare that the contents of this thesis are my original work and have not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University Supervisors.

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ABSTRACT

Myrica salicifolia Hochst ex A. Rich (Myricaceae) is a tree whose root extracts are drank by Masai warriors to prime themselves for battle. In humans the extract is reported to produce detachment from the external environment, feelings of invincibility, irritability, aggressiveness, overreaction to extraneous sounds and a tendency to keep a posture for a long time.

The objective of this work was to explore the usefulness of this plant by defining its pharmacological profile in laboratory animals. To this end the plant extract was put through a battery of neuropsychopharmacological tests and an examination of its systemic pharmacological and toxicological effects.

Tested on gross behavioural effects in rats and mice, *M. salicifolia* root extract proved to be a central nervous system (CNS) depressant which did not cause sleep even in high doses. The depressant activity was characterised by reduction in spontaneous and motor activity, muscle relaxation, palpebral ptosis and catalepsy. The depressant effect was further reflected by the potentiation by the extract of the hypnotic effect of three different CNS depressants, diethyl ether, ethanol and pentobarbitone.

It also reduced the open field behaviour (a measure of spontaneous activity) in the mouse and impaired motor performance in the mouse as measured by the traction and chimney tests.

M. salicifolia extract was inactive on three screening tests for neuroleptic activity viz: reduction of apomorphine-induced stereotypy, amphetamine-induced stereotypy and amphetamine lethality in aggregated mice. It had some activity on a fourth screeening test: reducing amphetamine hypermotility in mice. On two tests for antidepressant activity - reserpine induced ptosis and the behavioural despair test, the extract was inactive but on a third test, prolongation of duration of amphetamine stereotypy it was marginally active.

Despite its central depressant activity, M. salicifolia extract was inactive against convulsions induced by caffeine, bemegride and 3-mercaptopropionic acid. It also lacked central anticholinergic activity but it showed potent morphine-like analgesic activity on two models of pain in the mouse: the hot plate and the tail pressure tests. The extract also had potent hypothermic and antipyretic effects.

The extract produced contraction of the intestines *in vitro* but reduced gut motility *in vivo* when administered intraperitoneally but not when administered orally. It also reduced blood pressure in the anaesthetised rat but has no effect on the isolated rabbit heart. The extract lacked diuretic activity, antiinflammatory activity, local anaesthetic activity and activity on the neuromuscular junction. It however produced contraction of the rat uterus and the guinea pig trachea *in vitro*.

The toxic effects of the extract were characterised by peritonitis when administered intraperitoneally, paralysis of the hindlimbs and ulcerogenic activity in rats. The median lethal dose (LD_{50}) in mice was found to be 248 ± 22mg/kg.

The root of *M. salicifolia* contains phenolic compounds, terpenes and tannins but no alkaloids. The active component(s) have not been identified but the extract has been standardized by spectrophotometric methods. A lipid-soluble phenolic compound was isolated but not in adequate quantities to allow for pharmacological evaluation.

It was concluded that *Myrica salicifolia* root was a non-hypnotic CNS depressant with muscle relaxant, analgesic, hypothermic and antipyretic properties and potential but still undefined psychotherapeutic applications.

Chapter One

INTRODUCTION

1.1 General Introduction

1.1.1 Psychoactive drugs of plant origin

The use of drugs that affect the mind has been widespread in all cultures from ancient times. In ancient Greece, Homer in his Odyssey describes Helen of Troy dispensing a psychoactive drug in wine at a party to dispel the sorrow of her guests who were depressed over the non-return of Odysseus from the Trojan war (Caldwell 1978). Hippocrates, the father of modern medicine, administered mandrake (*Mandragora officinarum*) which contains centrally active tropane alkaloids to those of his patients who were depressed with suicidal tendencies (Caldwell, 1978). The Sumerians were aware of the analgesic and euphoriant properties of opium 4000 years ago and called the opium poppy (*Papaver somniferum*) 'the plant of joy' (Schultes 1978). Other psychoactive plants with long histories of usage by humans are *Cannabis* which has been cultivated for 8000 years, tobacco which features in the Mayan creation myths, and peyote which has been found in 4000 year old archeological sites in Texas (Schultes, 1978).

Naturally occuring psychoactive substances are difficult to classify into the conventional pharmacological categories and the broad classification used by Schultes (1978) is probably best viz. Excitantia, Euphorica, Hypnotica, Inebrianta and Phantastica.

1.1.1.1 Excitantia

These are substances that produce mental stimulation, often but not always followed by depression.

The caffeine-containing plants are the most important both from the numbers of people using them on a regular basis and from their economic importance. The most important are tea (*Camellia sinensis*), a native of China where it has been used since well before 600AD, coffee (*Coffea arabica*, *C.canephora* and *C. liberica*) a native of Abyssinia, and cacao (*Theobroma cacao*) which is a native of the Amazon basin and contains theobromine in addition to caffeine. These three plants are cultivated widely in the tropics and sub-tropics and are used as mild stimulants all over the world mainly as beverages.

Cola nuts (from the tree *Cola nitida*) contain 2% caffeine and theobromine.

In West Africa, where they are produced they are chewed for their stimulant effects, while in the rest of the world they are used to make carbonated cola drinks like Coca Cola (Schultes 1978).

The other caffeine containing plants are only important in South America where they are indigeneous. These are guarana (Paullinia cupana), yoco (P. yoco), yerb mate' (Ilex paraguariensis) and guayusa (I. guayusa).

Next in importance to the caffeine-containing plants is tobacco (Nicotiana tabacum) which is mainly smoked in cigarettes, cigars and pipes but is sometimes chewed or used as snuff. It is indigeneous to North America and was introduced to the rest of the world, initially to Europe by the crew of Christopher Columbus late in the 15th Century. The active constituents of tobacco are still a subject of debate but the leaves contain the pyridine alkaloids nicotine (0.6-9%) and nornicotine and the aromatic principle nicotianin which is responsible for the 'flavour' of tobacco leaves (Schultes, 1978).

Another stimulant plant is Khat (Catha edulis) a native of East Africa whose effects are produced when the fresh leaves and young twigs are chewed.

The effects include excitation, euphoria, hyperactivity and suppression of hunger and sleep. Habitual Khat chewers develop psychological dependence, aggressive behaviour, paranoid ideas and personality disorders. The active compounds are d- norpseudoephedrine and cathinone (Guantai 1982, Kalix 1984).

The nuts of the betel palm (Areca catechu) are widely chewed in Southern Asia, Indonesia, and the Pacific. Before chewing the nuts are mixed with some flavourings, cloves, tamarinds, nutmegs (which are psychoactive) and coconut. This produces mild stimulation and euphoria effects which have been ascribed to arecoline and other alkaloids in the nut (Schultes 1978)

1.1.1.2 Euphorica

These substances produce a feeling of well-being. It is noteworthy that the two plants in this group coca (Erythroxylon coca) and opium (Papaver somniferum) contain potent pain killers and produce psychological and physical dependence (addiction) on chronic use.

Coca is indigenous to South America where the inhabitants have chewed the leaves for the last 2500 years. Coca chewing which over the years had attained great religious significance suppressed hunger and fatigue and increased

vigour enabling the South American Indians to work long hours in the rarefied air of the Andes Mountains. (Caldwell 1978). The active component of coca leaves, the alkaloid cocaine was once widely used as a local anaesthetic but is now largely restricted to surface anaesthesia of the cornea and respiratory passages. In low doses cocaine causes euphoria and motor stimulation while chronic use leads to delusions, hallucinations and paranoia (Abel 1974, Turner and Richens 1978). Cocaine causes physical dependence and is the subject of a major illegal trade.

Opium is a brown, amorphous gum formed from air-drying the latex of the unripened capsule of the opium poppy (Papaver somniferum) which is native to the Near East. It contains 25 alkaloids the most important of which are morphine, a potent narcotic analgesic; codeine, an antitussive sedative and weak analgesic; and papaverine, a smooth muscle relaxant.

The medicinal and psychotropic effects of opium have been known for a long time - it was used by the Sumerians 4000 years ago, in Egypt by 1500 BC and in India before Buddha's time. It was introduced to Western medicine by Paracelsus in 1521 (Schultes 1978).

Opium and its alkaloid morphine produce a feeling of intense euphoria and well-being. The pursuit for this desirable state has resulted in morphine and its derivative heroin becoming major drugs of abuse.

1.1.1.3 Hypnotica

These are substances that induce a dreamy or somnambulistic state. There is only one example, kavakava, the dried root of the shrub, Piper methysticum (Schultes 1978). The root is used as an intoxicant in the South Pacific Islands such as Fiji either by drinking the infusion or chewing the root after softening by adding water. It produces a somnambulistic dreamy state characterised by euphoria, loquaciousness and sometimes sleep. Reason and the use of limbs are not imparied. The root contains no glycosides or alkaloids and the activity is due to the resins dihydrokawain and dihydromethysticine. (Schultes 1978).

1.1.1.4 Phantastica

These are substances that induce some kind of hallucinations. A very large number of plants have hallucinogenic effects and only the most important need be mentioned.

Amanita muscaria is a hallucinogenic mushroom that is common in the north temperate zone of Europe, Asia and America. It has been used in many magico-religious rituals either by swallowing the mushroom-or because the active principles, pass through the body unmetabolised - drinking the urine of intoxicated individuals. The effects vary from person to person but they are characterised mainly by twitching, trembling, limb convulsions, numbness of the feet, euphoria, dancing and visual hallucinations, (Schultes 1978).

Other hallucinogenic mushrooms are from the genera Conocybe, Panaeolus, Psilocybe and Stropheria. These grow in Mexico where they have been used in magico-religious ceremonies for the last 3000 years. The active principles are psilocybin and psylocine which are analogues of tryptamine. They produce visual and auditory hallucinations, physical and mental depression and distort space and time perception. (Schultes 1978).

The roots of the shrub Tabernanthe iboga have been used in Gabon and Congo in traditional religious ceremonies (Schultes 1978). The root produces central stimulation and auditory and visual hallucinations. These effects have been ascribed to ibogaine and a dozen other indole alkaloids which are found in the root. (Schultes, 1978; Downing 1964).

Another important hallucinogen is the Mexican cactus, Peyote (Lophophora williamsii) which has been used as an intoxicant for the last 4000 years. The dried tops, called mescal buttons are swallowed whole and produce auditory, tactile, gustatory and olfactory hallucinations, sensations of weightlessness and alteration of time perception (Castaneda 1972, Schultes 1978). Peyote contains over 30 alkaloids the principal one being mescaline.

The most widely used hallucinogen is Cannabis, mainly C. sativa but also C. indica and C. rideralis. The flowering tops and leaves or the resin from the pistillate flowers (hashish) are usually smoked or sometimes eaten or drunk for their psychotropic effects. These include euphoria, sensory enhancement, reduction of emotional control, physical and mental relaxation, and a dreamy fantasy state. There is no impairment of memory, sensory acuity, intellectual ability or physiological responses even among heavy cannabis users but transient psychotic episodes and problems of social adjustment have been observed (Cohen 1979).

While dozens of cannabinoids have been identified in cannabis the psychic activity has been attributed to Δ^9 Tetrahydrocannabinol (THC). THC is one of a number of compounds obtained from cannabis with potential therapeutic applications:

THC and cannabidiol are effective in grand mal epilepsy; THC is a bronchodilator and could also be used as a prenaesthetic agent; and cannabinoids reduce intraocular pressure and could be used to treat glaucoma (Cohen 1978).

Many solanaceous plants have been used all over the world for their hallucinogenic effects. The active principles are the tropane alkaloids mainly hyoscyamine and scopolamine. These include Atropa belladonna, Hyoscyamus niger and Mandragora offinarum used in medieval Europe; Datura arborea, D aurea and D. candida among others in South America; Datura metel in India and China; Latua pubiflora among the Indians of Chile; Datura stramonium among the Indians of North America and Datura inoxia and D. ceratocaula among the Mexican Indians. (Schultes 1978)

1.1.1.5 Inebriata

These are drugs that produce initial stimulation of the central nervous system followed by depression that may lead to suppression of normal mental functions.

The most important in this group and indeed among the most widely used psychoactive agents is ethyl alcohol (ethanol).

Alcohol has been used since prehistoric times and is produced from the fermentation of sugars by moulds or bacteria but mainly by yeasts, most commonly strains of the ascomycete, Saccharomyces cerevisiae. Alcohol production therefore requires two plants - the sugar source and the fermenter.

The sugar is usually produced by enzyme hydrolysis of starch - diastase in germinating cereals, ptyaline from human saliva or fungal amylases. The substrate for alcohol production could therefore be either a sugar-rich fruit or a starch-rich cereal - maize, rice, barley or vegetable like the banana. The ubiquitousness of yeast and sugar and starch-rich crops explains the universality of alcohol. Because alcohol inhibits the activity of yeast at a relatively low concentration the manufacture of liquors with a higher alcohol content than wine and beer had to await the discovery of distillation.

In conclusion, it is interesing to note the application of psychoactive drugs in almost all cultures to two very diverse needs - to fight boredom and to complete religious ceremonies. It is to create variety in otherwise dull lives that cannabis has been used over the centuries and heroin and cocaine and just a decade or two ago LSD, captured so much attention in the West.

Religious applications are equally evident. The use of psilocybe in Aztec religious ceremonies 600 years ago and of wine in the modern day Catholic mass are but two examples of the use of psychotropic drugs to facilitate the religious experience.

1.1.2 Psychotropic effects of Myrica salicifolia Uses in traditional medicine

Myrica salicifolia Hochst, ex A. Rich, the subject of this work has a number of documented applications in traditional medicine in Eastern Africa. Kokwaro (1976) reported the use of the roots for stomach troubles, headaches and as a vermifuge; the bark for cough and toothaches and the leaves for skin diseases. Watt and Breyer-Brandwijk (1962) have reported the use of the bark for malaria, the leaves for diarrhoea in the goat and the roots for indigestion and nerve conditions.

During the present work, information obtained from interviews of traditional medicinemen and other people well steeped in traditional lore indicated that the much feared Masai Warriors, the 'morans' use aqueous extracts of M. salicifolia roots to prime themselves for battle.

Accounts of the setting and the experience of the users of M. salicifolia root extracts were obtained from elderly people in Narok district who had been morans in their youth. It was not possible to interview active morans as they are hostile and inaccessible.

Traditionally, after circumcision young Masai men left home to live in the bush away from the rest of the community for several years. While there they served as guardians of the community and spent their time on cattle raiding expeditions, lion hunts, dancing and decorating themselves. In preparation, for these missions they would go to their fathers, get a few bulls and engage in lengthy orgies of feasting.

While the meat was cooking the root bark of M. salicifolia plus a few other plants were boiled in large containers and the extract put away to cool, usually in a cavity in a rock. After eating the meat to satisfaction the warriors would individually walk to where the root extract was placed and drink some using a bull's horn. This had the immediate effect of stimulating the appetite and the warriors would return to the meat eating place.

Within days of starting the feast the psychotropic effects of M. salicifolia root brew started to show in some of the warriors.

This was characterised by withdrawal into themselves, emotional incontinence - quickness to anger, unjustifiably sharp reactions to extraneous sounds, frenzies of involuntary shivering of the limbs and bouts of sobbing. Because of the tendency to pick fights (often bloody) with their colleagues, those who were most affected were restrained by being tied to trees by the others and fed while there. Once tied they exhibited catalepsy (keeping to the positions they were placed), stared blankly into space, and had urinary incontinence and fits of aggressive behaviour presumably directed at the victims of the proposed mission.

On lion hunts, those who were most affected were usually left behind for their own safety for otherwise they were incautious in their attack (attributed to their lack of fear) and formed the bulk of the victims of a cornered lion.

The 'moran' are also said to be intolerant to the smell of women and if a woman inadvertently passed near them they reacted by holding their noses. This is also attributed to the psychotropic effects of M.salicifolia and the other plant extracts they took.

In addition to its use as a psychotropic agent by the warriors, the root of M. salicifolia is used in much smaller quantities as a tonic in bone soup by other age groups.

Unique though the use of a psychotropic plant to enhance the martial spirit of warriors may seem, it has at least one historical antecedent, albeit in a different culture. The Scandinavian Viking Berserkers who terrorised the European West Coast ten centuries ago are said to have owed their conduct, and their name, to the hallucinogenic mushroom Amanita muscaria. They either ate the mushroom or drunk its active principle after fortification through human passage (Caldwell 1978). In the sense that it is associated with the use of psychoative agents the legendary militancy of the Vikings and of the Masai warriors seems not too unlike the 'Dutch courage' produced by consumption of moderate quantities of alcohol.

1.2. The Family Myricaceae - Review of the Pharmacology and Phytochemistry

1.2.1 The pharmacology of the Myricaceae

The fruit of *Myrica gale* has been used in beer making in Europe for a long time as a flavouring agent although it may also have a preservative effect as well (Malterud 1982). The preservative effect is suggested by the fact that both the fruit extract and dihydrochalcones extracted from the fruit have bacteriostatic and fungistatic activity (Malterud and Faegri 1982). The extract of the bark of *M. gale* also suppresses the development of a bacteriophage of *Pseudomonas pyocyanea* at a concentration that has no effect on the host. This effect has been attributed to the tannin content of the extract (Chantrill *et al* 1952). The leaf extract of *M. gale* has also been used to stabilise pharmaceuticals like monoglycol salicylate in adhesive pastes (Nitto Electric Industrial Co. 1984).

The bark of *M. rubra* has been used in Chinese traditional medicine in the management of burns presumably because of the escharotic effect of the tannins (Siang 1983).

A 1% aqueous extract of the bark of M. rubra was inactive against pathogenic fungi of the family Trichophyton (Ito and Ota 1952). The hydrolysis products of the flavonoids of M. rubra inhibited the growth of Bacillus subtilis, B. megaterium and Escherichia coli and have been patented for use as air fresheners (Antsune 1976).

The root extract of M. cerifera has been reported to be an effective medicament for arthritic conditions (Burnett 1982) and also when given in miniscule quantities to normal humans over a 5½ week period to increase plasma bilirubin, gastric fasting contents, emptying time and acidity (Hepburn et al 1950). Extracts of M. cerifera have also been reported to increase biliary secretion in rats (Pirtkien et al 1960) and to be carcinogenic when injected subcutaneously in rats, the later effect being attributed to the tannin content (Kapadia et al 1976).

Tannins from some species of the genus Myrica have been isolated and found to be useful metabolic regulators in animals (Nippon Shinyaku Co. 1983).

1.2.2 The Phytochemistry of the Myricaceae

Essential oils are apparently ubiquitous in the Myricaceae family - they have been isolated from the leaves of *M. pensylavanica* (Tattje and Bos 1971), *M. phanerodonta*, *M. pubescens* and *M. mexicana* (Collins and Halum 1973) *M. macfarlanei*, *M. heterophylla*, *M. cerifera*, *M. gale* *M. pensylvanica* and *M. pusilla* (Halum and Collins 1973). The chemical composition of these essential oils has been suggested to have chemotaxonomic importance (Collins and Halum 1973, Halum and Collins 1973). Besides essential oils other compounds reported from the Myricaceae family are flavonoids in the leaves and fruits; and flavonoids, triterpenes and tannins in the stem and root bark.

The following compounds have been reported from *M. gale*: myricetin and quercatin glycosides from the leaves (Bodalski and Rzadkowska - Bodalska 1969); dihydrochalcones from the fruits (Tahsin *et al* 1978, Malterud and Faegri 1982); chalcones and other flavonoids from the leaves and fruits (Anthonsen *et al* 1971), Malterud *et al* 1977); galeon and hydroxygaleon (Malterud *et al* 1976); myricanone from the stem bark (Malterud 1981); ursolic and oleanolic acids (Borovkov and Beloca 1962); and tannins (Chantrill *et al* 1952)

From M. rubra the following compounds have been reported: myricetin and myricitrin (Chao-1 and Kuang-Yueh 1947, Shimizu et al 1952); myricatin and prodelphinidin gallates (Nonaka et al 1983); myricanol, myricanone and myricanol glucoside (Inoue et al 1984); and tannins (Ito and Ota 1952, Siang 1983).

The following compounds have been reported in M. cerifera; myricardiol, taraxerol, taraxerone and myricitrin (Paul et al 1974); and flavonoids (Rodriguez et al 1974).

Other compounds reported are myriconol (Krishnamoorthy et al 1963) and proanthocyadins (Krishnamoorthy and Seshadri 1966) from M. nagi; taraxerol, myricardial, and β'- sitosterol (Agarwal et al 1963) from M. esculenta; sterols and triterpenes from M. pensylvanica (Hooper and Chandler 1984); and myricardiaol, taraxerol and taraxerone from M tomentosa (Matyukina et al 1968)

1.3. Myrica salicifolia

1.3.1 Introductory

Myrica salicifolia Hochst, ex A. Rich belongs to the genus Myrica Linn. Myrica is one of the two genera in the family Myricaceae - the other is Comptonia (Halum and Collins 1973) and consists of 50 species most of which are distributed widely in the Northern Hemisphere. Of these, 16 species occur in Africa (Hutchinson 1917) but only three have been reported in Kenya: Myrica kandtiana Engl. M., meyeri-johannis Engl. and M. salicifolia (East African Herbarium, Nairobi). M.salicifolia is known by a number of vernacular names in Kenya viz. Olkitoloswa (Masai), Mukikia, Muthogoya (Kikuyu), Kibogen (Marakwet) and Kabuneto (Kipsigis).

1.3.1 Nomenclature

Myrica salicifolia was first described as a species by A. Richard in 1851. Later Engler separated Myrica kilimandscharica Engl. from M. salicifolia on the character of the male flowers being single in the axils of the bracts.

Staner and Lebrun (1933) also described differences in the shape and size of leaves and branch lenticellation between M. salicifolia and M. kilimandscharica. However a subsequent review (Anon. 1940) considers that since "the shape, size and dentation of the leaf (of M. salicifolia) is very variable" this character does not justify classification of M. kilimandscharica as a separate species. The two species are therefore considered synonymous and are referred to as Myrica salicifolia Hochst, et A. Rich.

For the purposes of this work the plant has been referred to as Myrica salicifolia, the name suggested by Anon. (1940) and adopted by the East African Herbarium and Dale and Greenway (1961).

However Watt and Breyer-Brandwijk (1962) refer to the plant as M. kilimandscharica while Kokwaro (1976) lists them as two different species.

It should however be noted that this lack of agreement on nomenclature extends to the whole family - there are a lot of differing opinions on the generic and species delimitations within the Myricaceae family because of the wide variation even within species (Halim and Collins 1973).

1.3.3 Botanical description

M. salicifolia is a shrub or tree which grows to a height of about 15 metres. (Plate 1 and Fig. 1) It has grey-brown branchlets which are puberculous - glandular when young and glabrous when mature. The bark is rough and dark blackish - brown in colour. The leaves are undulate or dentate - serrate in the upper half or two-thirds and are oblong to elliptic - lanceolate. They are usually 4 - 13 cm long and 2 - 4.5cm wide, with small golden glands on both surfaces but especially below. The apex of the leaf is obtuse and often mucronate and the base is unequally sided. The patiole is 0.6 - 1.3cm long.

The flowers are yellow, fragrant and dotted with oil glands. The male spikes are axillary, crowded and very densely flowered and grow upto 2.5cm in length. The rachis has a yellow tomentose. The fruiting spike is upto 4 cm long. The fruit is warted, ellipsoid or globular in shape and is 40mm in diameter. It is purplish in colour and often has a white waxy covering. (Dale and Greenway 1961).

1.3.4 Geographical Distribution

M. salicifolia grows in montane forests at an altitude of 2000-3000 metres above sea level.

It is found in several East and Central African countries such as Burundi, Ethiopia, Kenya, Malawi, Rwanda, Tanzania Uganda, and Zaire (East African Herbarium, Staner and Lebrun 1933).

In Kenya it is common in the forests of the Aberdare ranges and around Mt. Kenya. It has also been collected in the Taita Hills, Marakwet Hills, West Mau, Longonot and on the forests around Mount Elgon (East African Herbarium, Dale and Greenway 1961).

1.4 Introduction to the present work

It would appear from the interviews with former warriors that to understand the legendary ferocity of the Masai warrior cadre, the 'moran', one has to look beyond the spartan, celibate discipline and the long training they undergo. For the ex-'moran' assert, to a man, that the use of *M. salicifolia* contributes in no small measure to their aggressiveness.

Literature survey indicates that there is no reported work on any aspect of the phytochemistry or pharmacological effects of *Myrica salicifolia*. In a preliminary study (Kofi-Tsekpo *et al* 1985) we have shown that *M. salicifolia* extract affects the gross behaviour and prolongs pentobarbitone sleeping time in mice.

The aim of the present work was to investigate the pharmacology and toxicology of *M. salicifolia* extract and in particular to ascertain its effects on a battery of psychopharmacological tests in animals that would shed light on the reported human effects.

Preliminary phytochemical screening of *M. salicifolia* was also included in the study.

Plate 1- Myrica salicifolia growing in Aberdares ranges.

a: part of branchlet from ♀ plant with young fruits (× 1). b: branchlet from ♀ plant (× 1). c: part of ♀ 'catkin' (× 8). d: fruit (× 7). e: branchlet from ♂ plant (× 1). f: part of ♂ 'catkin' (× 4).
a-d: from Drummond & Hemsley 1619; e & f: from Drummond & Hemsley 2901.

Fig 1: Leaves and flowers of Myrica salicifolia

(from Dale and Greenway 1961)

MATERIALS AND METHODS

Chapter Two

PHYTOCHEMICAL INVESTIGATION

2.1 Introduction

Preliminary phytochemical screening of *Myrica salicifolia* roots had indicated that it does not contain alkaloids (Kofi-Tsekpo *et al* 1985). This is interesting because the bulk of psychoactive agents of plant origin are alkaloids e.g. morphine, cocaine, ibogaine, nicotine and caffeine. A more rigorous phytochemical screen was therefore conducted.

2.2 Collection and preparation of plant material

Collection:

Roots of *Myrica salicifolia* were dug up in May, 1985 at Matindiri off the Tumaini - Ol Kalau road in Nyandarua District of Central Kenya. This is part of the Aberdare range with an altitude of 2500 metres and is 150 kilometres from Nairobi. The authenticity of the plant was established by the East African Herbarium, Nairobi. Pressed voucher specimens were kept at the Kenya Medical Research Institute. The bark was then removed from the roots and stored in a deep freeze at $-50^{0}C$ until used.

Extraction:

The root bark (1kg) was cut into small pieces and extracted with four successive 2 litre aliquots of warm methanol.

These aliquots were bulked and evaporated over a rotary evaporator (Buchi, Switzerland) at 70^0C. The final traces of water were removed by azeotropic evaporation with absolute ethanol. The extract was a light brown flaky powder. The dry extract was then stored in an stoppered flask in a refrigerator. Sixty six (66) grams of extract was obtained. This has been refered to as Myrica salicifolia extract in the rest of the text.

2.3 PHYTOCHEMICAL SCREENING

2.3.1 Tests for alkaloids

0.5g of the extract was boiled with 10ml of acid alcohol in a boiling tube for 1 minute and cooled. To the supernatant (1ml) was added 3 drops of Dragendorff's reagent. A precipitate indicates the presence of alkaloids (Brain and Turner 1975).

b) A TLC plate on which M. salicifolia extract was spotted was developed in Isopropanol:Methanol:Ammonia (85:15:10). After drying the plate was sprayed with Dragendorff's reagent. Orange spots indicate alkaloids (Waldi 1965)

2.3.2 Test for anthraquinones

0.1g of the extract was heated with 10ml water for 5 minutes filtered while hot, cooled and extracted with 10ml carbon tetrachloride.

The carbon tetrachloride layer was washed with 5ml water and shaken with 5ml dilute ammonia solution.

Another 0.1g of the extract was heated for 10 minutes with 10ml ferric chloride solution and 5ml hydrochloric acid. It was filtered hot, cooled and treated as above with carbon tetrachloride. A pink to cherry-red colour in the ammoniacal layer in the first test indicates free anthraquinones. No colour change in the first test but red colour in the second test indicates the presence of bound anthraquinones only. If both free and bound anthraquinones are present both tests will be positive but the second would be more intense. (Brain and Turner 1975)

2.3.3 Test for saponins

A small amount of the extract was shaken with distilled water in a test tube. Frothing lasting over 30 minutes indicates the presence of saponins. (Brain and Turner 1975, Sim 1967)

2.3.4 Tests for phenolics

a) To a methanolic solution was added a few drops of 1% aqueous ferric chloride solution. Blue green or black coloration is indicative of phenolics (Harborne 1973).

b) A TLC plate on which M. salicifolia extract was spotted was developed in Isopropanol:Methanol: Ammonia (85:15:10). After drying the plate was sprayed with a fresh aqueous mixture of 1% ferric chloride and 1% potassium ferricyanide. Blue spots indicate phenolics (Harborne 1973).

c) A similarly developed TLC was sprayed with Folin Ciocalteau Phenol reagent (Sigma) diluted three times with water. Blue spots were indicative of phenolics (Harborne 1973).

2.3.5 Tests for flavonoids

a) To an ethanolic solution of the extract was added magnesium filings followed by concentrated hydrochloric acid dropwise. The various classes of flavonoids (flavones, flavonols, flavonones and chalcones) produce a range of colours ranging from green, yellow to red. (Geissmann 1955).

b) A TLC plate on which the extract was spotted was developed in Isopropanol: Methanol: Ammonia (85:15:10). After drying, it was sprayed with 1% aluminium chloride in ethanol and viewed under uv light. Yellow-green fluorescence indicates flavonoids. (Anon 1973b, Harborne 1973, Waldi 1965)

c) To an aqueous solution of the extract was added concentrated sulphuric acid dropwise. Flavonoids are indicated by the solutions changing to particular colours viz: flavones and flavonols turn deep yellow, flavonones turn orange or crimson, chalcones and aurones turn deep red, crimson or magenta (Geissmann 1955).

2.3.6 Tests for Tannins

a) An aqueous solution of the extract was treated with a 1% aqueous solution of gelatin. A white precipitate indicates tannins (Anon 1973a).

b) An aqueous solution of the extract was treated with an aqueous solution of bovine albumin. A white precipitate indicates tannins (Anon 1973a).

c) An aqueous solution of the extract was treated with ferric chloride solution (1%). Dilute sulphuric acid was then added dropwise. Tannins turn blue-black on adding ferric chloride solution but the colour disappears to give a yellowish-brown precipitate on adding dilute sulphuric acid (Anon 1973a).

d) An aqueous solution of the extract was treated with an aqueous solution of ammonium molybdate. A brown precipitate indicates tannins (Naik et al 1980).

2.3.7 Test for steroids

A developed TLC plate was sprayed with a saturated solution of antimony trichloride in water - free chloroform and the plate heated at 100°C for 5 - 10 minutes. Brown spots indicate steroids (Anon 1973b, Waldi 1965).

2.3.8 Test for Cardiac Glycosides

A developed TLC plate was sprayed with a mixture of equal volumes of 2% methanolic 3,5 - dinitrobenzoic acid and 2N aqueous potassium hydroxide (Kedde Reagent). Violet spots indicate cardiac glycosides. (Anon 1973b, Sim 1967, Waldi 1965).

2.4 THIN LAYER CHROMATOGRAPHIC (TLC) EXAMINATION OF M. SALICIFOLIA EXTRACT

2.4.1 Preparation of TLC plates

A slurry was made using silica gel for TLC (G - UV_{254}, Machery Nagel) in a pestle and mortal to a suitable consistency with distilled water. This slurry was poured into the feed chute of a Camag Spreader (Plate 2) and glass plates pushed through at constant rate with the adjustment made to give a coating of thickness 0.3mm. The plates were allowed to set, kept horizontally in a metal rack to dry and 24 hours later activated by placing in an oven at 120^0C for 30 minutes.

2.4.2 Spotting and development

M. salicifolia extract was spotted at 2cm intervals on the TLC plates using capillary tubes. To avoid spots spreading each application was allowed to dry before making another one.

The plates were developed in clean 20 x 10 x 20cm glass TLC tanks lined with Whatman chromatography paper in which 100ml of the solvent system had been poured and allowed to equilibrate.

Detection:

After development the plates were removed from the tank and placed vertically to dry and then examined under visible light, short (254 nm) and long (365nm) uv light in a UV irradiating chamber (Model Sp-600, Toyo Kagaku Sangyo Co. Ltd, Japan).

The plates were also visualized by spraying with various spray reagents - Dragendorff's, Antimony trichloride, ferric chloride - potassium ferricyanide, folin - ciocalteau, Anisaldehyde etc.
Spots so visualized were marked and the Rf values (ratio of the distance moved by the spot to that moved by the solvent front) calculated.

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2.5 COLUMN CHROMATOGRAPHY OF M. SALICIFOLIA EXTRACT

Silica gel for column chromatography (E. Merck, 70-230 mesh A.S.T.M.) weighing 200g was packed under petroleum ether (60-80) in a 4cm diameter glass column. Ten grams of M. salicifolia root methanolic extract was adsorbed in 5g silica gel using a small amount of methanol and added to the top of the column and covered with fine purified sand. The column was then eluted with petroleum ether (60-80), mixtures of petroleum ether and chloroform, chloroform, mixtures of chloroform and methanol, and methanol in that order. Eluates of 250ml volume were collected and evaporated in vacuo with a rotary evaporator and spotted on TLC plates which were developed. Those eluates with identical TLC profiles were bulked.

2.6 Quantitation of M. salicifolia extract

The root extract of M. salicifolia was dissolved in methanol at a concentration of 10µg/ml, and scanned on a UV - visible Spectrophotometer (Beckman Du-6). The maximal absorbance (λ max) occured at a wavelength of 211nm. The absorbances of 5, 10, 15, 20 and 25µg/ml of the extract in methanol were measured at this wavelength. The absorbances were plotted against the concentration and used to calculate the absorbance of a 1% solution.

2.7 Isolation of a compound from M. salicifolia roots.

When the M. salicifolia root extract was eluted from the column, one of the eluates - with petroleum ether - was found to have a single spot when chromatographed on TLC. The semi -solid obtained on evaporating petroleum ether was insoluble in ethanol or methanol and was crystallized in methanol. A smear of this semi-solid was applied on sodium chloride cells and its infra-red (IR) spectrum determined on a infra-red spectrophotometer (Pye Unicam SP1000).
The isolated compound was also scanned on a UV spetrophotometer (Pye Unicam SP800) in petroleum ether solution.

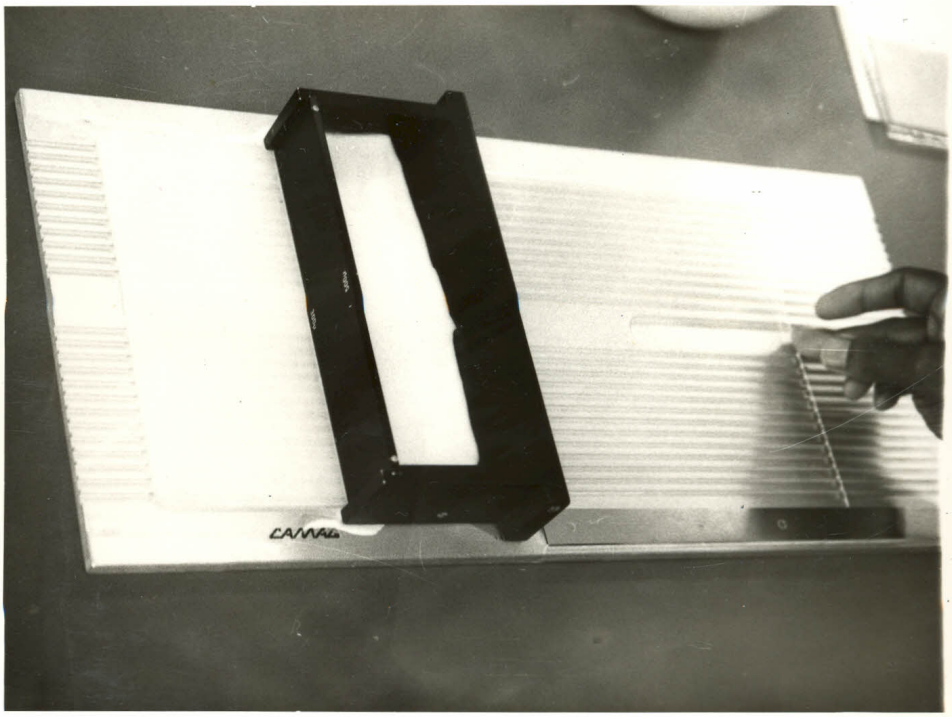


Plate 2 - Camag spreader for Preparing Thin Layer Chromatography plates.

PREPARATION OF PHYSIOLOGICAL SALT SOLUTIONS

QUANTITIES FOR 1 LITRE (g)

	LOCKE	TYRODE	KREBS-HENSELEIT	DE JALON
NaCl	9.0	8.0	6.87	9.0
KCl	0.42	0.2	0.4	0.42
$CaCl_2$	0.24	0.2	0.28	0.06
$MgCl_2$	-	0.1	-	-
$MgSO_4$	-	-	0.14	-
$NaHCO_3$	-	0.05	-	-
KH_2PO_4	-	-	0.16	-
$NaHCO_3$	0.2	1.0	2.1	0.5
Glucose	1.0	1.0	2.0	0.5

Chapter Three

PHARMACOLOGICAL INVESTIGATION

3.1 Introduction

Since the reported human effects of *M. salicifolia* extracts are on the central nervous system (CNS) an investigation on the pharmacological basis of the effects in animals has to perforce concentrate on the CNS. These reported effects could conceivably be produced by agents from any of a number of psychopharmacological classes. The extract was therefore put through a battery of psychopharmacological tests to screen for neuroleptic, antidepressant, stimulant, anxiolytic and psychotomimetic activity. (The nomenclature used in this work for psychotropic drugs is that proposed by Usdin (1978) and which has gained wide acceptance (Petracek *et al* 1978, Berger 1978, Itil 1978, Cohen 1978).

Since other centrally acting drugs whose pharmacological effects are not primarily behavioural also have psychotropic effects, *M.salicifolia* extract was also screened for analgesic, anticonvulsant, antipyretic, antiparkinsonian and temperature regulatory effects.

In addition to CNS investigations, M. salicifolia was also studied on other physiological systems. This was done on the realisation that CNS effects cannot be divorced from effects on the rest of the organism. The effects investigated were those on the cardiovascular system, the gastro-intestinal tract, the genitourinary tract, the neuromuscular junction, inflammation and the peripheral nervous system.

In all tests a positive control was used. This was usually the prototype drug in that pharmacological group being tested and it served to validate the method. The dose used was that known to be effective from the literature and was usually established to be effective empirically before adoption.

For the bulk of the whole animal experiments M. salicifolia was used at a dose of 100mg/kg body weight. This was the dose found in the gross behavioural observation to produce maximal effects on the parameters used without producing deaths. Occasionally a dose of 200mg/kg was used as well.

3.1.1 Preparation of Drugs

For in vivo animal experiments, methanolic extracts of Myrica salicifolia roots were administered

orally or intraperitoneally (i.p)/saline (i.p) or /in distilled water (oral) at volumes of 5 or 10ml/kg body weight for mice and 5ml/kg body weight for rats. Control animals were administered a corresponding volume of 2% w/v gum acacia in water or saline. Reference drugs used as positive controls were dissolved in saline or suspended in 2% w/v gum acacia in saline or distilled water.

For in vitro experiments, M. salicifolia extract was suspended in saline and the supernatant used. For these preparations where particles could block capillaries such as isolated perfused rabbit heart and rat hind-quarters preparations the supernatant was rendered particle - free by passing through a membrane filter (Millipore Corporation Type HA, pore size 0.45μm).

3.1.2 Animals used

In tests involving chemoconvulsants, AJ strain female albino mice from the International laboratory for Research on Animal Diseases(ILRAD) Kabete were used, for carrageenan-induced paw edema and test for ulcerogenic activity, male Wistar strain albino rats from the Department of Pharmacy, University of Nairobi were used.

In all other tests, the mice were random bred Swiss strain albinos from Veterinary Laboratories, Kabete and National Public Health Laboratories animal houses and the rats were sprague - Dawley strain albinos from ILRAD or bred at the Kenya Medical Research Institute (KEMRI) animal house from the ILRAD stock.

Coloured guinea pigs from Veterinary Labs, Kabete and a locally bred strain of albino rabbits were also used.

The animals were kept in regulated temperature (20-22^{0}C) conditions in a natural light cycle, which at only 1^0 South of the equator is virtually 12 hour light/12 hour dark. Unless where otherwise stated the animals had free access to food and water. The experiments were conducted at room temperature 20-22^{0}C.

3.2 EFFECTS OF M. SALICIFOLIA EXTRACT ON THE CENTRAL NERVOUS SYSTEM

3.2.1 GROSS BEHAVIOUR CHANGES

A lot of information on the pharmacological activity of a new drug can be obtained by observing its effects on the gross behaviour of animals. The usefulness of this otherwise subjective study is vastly increased by using a standardized procedure with quantitative criteria. (Turner 1965, Malone 1977).

This involves monitoring a wide repertoire of behavioural, neurological and autonomic changes and it provides guidance on the direction of further research.

The method described by Turner (1965) was adopted. Groups of four mice (2 male, 2 female) were injected intraperitoneally with M. salicifolia extract in a wide range of doses: 10, 25, 50, 100, 200 and 400mg/kg body weight. Test animals were compared with gum acacia (2% w/v) treated controls 15, 30, 60 and 120 minutes after injection.

Records were made of changes in degree of the following characteristics using an arbitrary scale: alertness, motor activity, motor coordination, grip strength, body tone, pupil size, palpebral opening, respiratory rate and skin colour.

The presence of the following features was also recorded: stereotypy (frequent, mechanical repetition of a movement), passivity, vocalisation, irritability; pain, touch, startle, pinna and corneal reflexes; writhing and piloerection. M.salicifolia extract at 25, 100 and 200mg/kg body weight was also injected in pairs of male rats and the animals were evaluated in the same manner as the mice.

3.2.2 OPEN FIELD BEHAVIOUR

The open field apparatus is essentially a bare bounded arena where a naive animal is placed for a short length of time and observed for its spontaneous (unlearned) behaviour. Drugs that influence behaviour affect the animals explorative activity in this strange environment.

The open field apparatus and protocol used is a modification of that described by Jarbe and Johansson (1977). This was a 60 x 60 x 30cm wooden box open at the top with the floor lined with khaki paper divided into 16 squares (15 x 15cm) (Plate 3). Male Swiss mice weighing 18-28g were injected intraperitoneally with the test drugs and 30 minutes later placed at the centre of the open field arena and allowed to explore it for 3 minutes. Records were kept of the following measures:

Ambulation (horizontal movement) - number of squares crossed with all 4 feet.

Rearing (Vertical movement) - number of times the mouse stood on its hind feet.

Grooming - number of bouts the animal cleaned itself.

Defaecation - number of faecal boli

Urination - number of times the animal urinated.

The test drugs were M. salicifolia extract at 10, 25, 50 and 100 mg/kg body weight; the antidepressant amitriptyline (10 and 20mg/kg) the anxiolytic diazepam (10mg/kg) and the neuroleptic haloperidol (2 mg/kg). Control animals were injected with gum acacia 2% w/v. Each drug/dose was tested in groups of at least 8 mice.

3.2.3 SCREENING FOR NEUROLEPTIC ACTIVITY

Neuroleptics, formerly called the major tranquilizers (Berger 1978, Usdin 1978) are used for the treatment of psychoses. In animals they antagonise the stereotyped and nonstereotyped agitation induced by amphetamine and apomorphine. These effects show very good correlation with the clinical efficacy of the neuroleptics in the treatment of schizophrenia and are widely used as screening tests (Janssen and Van Bever 1978)

3.2.3.1 Amphetamine Hypermotility in mice

The method used was a modification of that of Yamada and Furukawa (1980). Male Swiss mice weighing 18-28g were injected intraperitoneally with the test drugs and 30 minutes later placed in the open field apparatus and scored for ambulation, rearings and groomings for 3 minutes as described above (Section 2.2.2).

On removing from the open field apparatus they were immediately injected with dexamphetamine sulfate 3.75mg/kg intraperitoneally, then 30 and 60 minutes later returned to the open field apparatus and scored as before. The test drugs were M. salicifolia extract (10 and 25 mg/kg), and haloperidol (2mg/kg). Controls were injected with 2% gum acacia. The dose of dexamphetamine used (3.75mg/kg) was that which was established empirically to cause significant hypermotility (Table 5) without appreciable stereotypy while that of haloperidol (2mg/kg) the lowest dose that appreciably reduced this amphetamine hypermotility.

3.2.3.2 Amphetamine lethality in aggregated mice

This test is based on a pair of discoveries: firstly, that the toxicity of amphetamine increases dramatically if mice are grouped compared with when housed singly (Chance 1946); and secondly that neuroleptics block this lethal effect of amphetamine in aggregated mice (Lasagna and McCann 1957, Burn and Hobbs 1958).

Male swiss albino mice in groups of 10 were injected intraperitoneally with M. salicifolia extract (100mg/kg), haloperidol (1mg/kg) or 2% gum acacia in saline. 1 hour later they were injected with dexamphetamine sulfate solution (50mg/kg) intraperitoneally and

kept in groups of 10 in small plastic cages (30 x 12.5 x 12.5 cm). They were observed for death at 6, 24 and 48 hours. Dead mice were replaced with uninjected mice to keep the number per cage at ten.

3.3.3 Amphetamine induced Stereotypy in Rats

Amphetamine in appropriate doses causes stereotyped sniffing, gnawing and licking in rats and mice. This stereotypy is antagonised by neuroleptics and has been widely used to screen for neuroleptic activity (Greenblatt *et al* 1978, 1980; Janssen and Van Bever 1978, Clark *et al* 1982, Fowler *et al* 1977a, 1977b). The method used to assess the effect *M. salicifolia* extract on amphetamine stereotypy is a modification of that of Meignen *et al* (1982).

Male sprague- Dawley rats in groups of six were injected with the test drugs intraperitoneally and 30 minutes later injected intraperitoneally with dexamphetamine sulfate 5mg/kg body weight. They were then placed individually in clean plastic cages and stereotypy scored every 30 minutes for 5 hours on the scale used by Creese and Iversen (1973): viz:

0 - asleep or stationary

1 - active

2 - ambulatory with bursts of sniffing or rearing.

3 - continuous sniffing or rearing with some ambulation.

4 - intensive continuous sniffing or rearing in one spot.

5 - continuous sniffing with bursts of gnawing or licking.

6 - continuous sniffing or licking of cage.

The test drugs were M. salicifolia extract (100mg/kg), and haloperidol (2mg/kg). Control animals were injected with a corresponding volume of 2% gum acacia.

3.2.3.4 Apomorphine - induced stereotypy in Rats

The method was that of Janssen et al (1965) as modified by Yamada and Furukawa (1980).

Female adult Sprague-Dawley rats were injected intraperitoneally with M. salicifolia extract, (100mg/kg) haloperidol (2mg/kg) or the vehicle, 2% gum acacia in saline and placed singly in clean plastic cages. 30 minutes later they were injected with apomorphine hydrochloride (5mg/kg) subcutaneously and returned to the individual cages. Stereotypy was scored every 10 minutes for 1 hour on the scale proposed by Costall and Naylor (1973) viz:

0 - normal behaviour

1 - exploratory activity, discontinuous sniffing

2 - continuous sniffing

3 - continuous sniffing. discontinuous licking biting and gnawing

4 - continous licking, biting or gnawing.

3.2.4 SCREENING FOR ANTIDEPRESSANT ACTIVITY

Three different tests were used to screen *M. salicifolia* extract for antidepressant activity. These were the amphetamine stereotypy test in rats, the reserpine ptosis test in mice and the behavioural despair test also in mice. The first two tests involve drug interactions - antidepressants reverse the effects of amphetamine and reserpine - while the third one tests against experimentally induced helplessness, the behavioural equivalent of 'depression' in the mouse.

3.2.4.1 Amphetamine stereotypy

The method used in assessing neuroleptic activity (3.2.3.3 above) was used but amitriptyline (10mg/kg) was used as the positive control instead of haloperidol.

3.2.4.2 Reserpine induced ptosis

A modification of the method of Jalfre *et al* (1982) was used.

Male Swiss albino mice in groups of 6 were used. The mice were injected with amitriptyline (5mg/kg), *M. salicifolia* (100mg/kg) or 2% gum acacia intraperitoneally 1 hour before intraperitoneally injection of 2.5mg/kg reserpine. Two hours later the degree of ptosis was assessed in all the mice on the scale:

0 - eyes full open

1/4 - eyes slightly closed

2/4 - eyes half way closed

3/4 - eyes almost fully shut

4/4 - eyes fully shut.

each eye was assessed separately on degree of palpebral opening.

3.2.4.3 Behavioural despair in mice

The method of Luttinger *et al* (1985) was adopted. Male Swiss mice weighing 17-27g were injected intraperitoneally with *M. salicifolia* extract (50 and 100mg/kg), amitriptyline (10 and 20mg/kg) and gum acacia as control. 80 minutes later the animals were immersed in 1 litre beakers containing 700ml tap water at 24^{0}C. After 2 minutes the mice were scored as either mobile or immobile every 30 seconds for 4½ minutes by an observer who was ignorant of the treatment administered. Mobility was defined as actively trying to get out of the water as opposed to just trying to remain afloat. Extraneous sounds were kept out by a background of continuous sounds provided by fans.

Total mobility/immobility scores over the 4½ minutes (10 in all) were summed up and compared with the control group.

3.2.5 TESTS FOR ANALGESIA

Two animal models for inducing painful stimuli were used to assess the analgesic activity of M. salicifolia extract. These are the hot plate test using a thermal stimulus and the tail pressure test using a mechanical stimulus.

3.2.5.1 Hot Plate test (Thermal)

The method used was a modification of that of Sofia *et al* (1977a). The flat surface of an inverted 2 litre flat bottomed flask in which hot water at 58 ± 1 ^{0}C was circulated with a heater stirrer (Techne) was used as a hot plate (Plate 4).

Eighteen hours before the test, male Swiss albino mice weighing 17-22g were individually placed on the hot plate and the control reaction time, the time taken for the mice to lick or flick their fore paws or jump off the hot plate was noted to the nearest 0.1sec. The method had been practised extensively to reduce the observer's reaction time. Only those mice responding within 10 seconds were used.

On the test day the mice were divided into three groups of ten. One group was injected with M. salicifolia extract (100mg/kg) intraperitoneally, the other with morphine hydrochloride (5mg/kg) and the last group with 2% gum acacia, the vehicle used to suspend the test drugs. Thirty minutes later the mice were re-exposed to the hot plate and the reaction time measured. To minimise tissue damage a cut-off time of 20 second was adopted.

3.2.5.2 Tail pressure method (Mechanical)

The method described by Shibata (1977) was used.

The same animals used for the hot plate test were used after 3 days. One hour after administration of drugs, the tail of the mouse 2-3cm from the tip was placed under the teflon tip of the Analgesy-Meter(R) (Ugo Basile, Varese, Italy) and the pressure gradually increased (Plate 5). The point at which the animal struggled was taken to be the reaction threshold. To avoid bruising the mouse tail the pressure exerted with the analgesy-meter was restricted to a maximum of 250g. The pressure was read on a scale reading from 0 to 25.

The test drugs were M. salicifolia extract (100mg/kg), morphine hydrochloride (5mg/kg) and 2% gum acacia (control)

3.2.6 EFFECT OF M. SALICIFOLIA EXTRACT ON CHEMOCONVULSIONS

3.2.6.1 Seizures induced by 3- mercaptopropionic Acid (3-MPA)

The method of Pieri *et al* (1981) was used. Female AJ strain albino mice weighing 20-22g were used. *M. salicifolia* extract (100 and 200mg/kg), diazepam (5 and 10mg/kg) and gum acacia (2%) were injected intraperitoneally and the mice placed singly in clean 1 litre beakers. 30 minutes later a 0.16% $^{v}/v$ aqueous solution of 3 - mercaptopropionic acid (3-MPA) was injected intraperitoneally at a volume of 0.5ml /20g mouse. This was made by diluting 0.08ml 3-MPA (liquid) to a volume of 50ml with distilled water and corresponded to a dose of 48.8 mg/kg 3-MPA. The mice were then returned to the beakers and observed for 30 minutes for the occurrence of

a) running or jumping fits
b) clonic seizures - twitching of fore and hind limbs
c) tonic seizures - extension of fore and hindlimbs
d) emprosthotonus - spastic bending of the body forwards.
e) loss of postural control and
f) death.

The test drug was deemed effective if it protected the animal from tonus: emprosthotonus and fore and hind-limb extension.

3.2.6.2 Bemegride induced convulsions

Female AJ strain albino mice weighing 20-26g were used.

They were injected with M. salicifolia extract (100mg /kg), diazepam (10mg/kg) or gum acacia and placed in clean 1 litre glass beakers. 30 minutes later the mice were injected with bemegride (20mg/kg) solution in saline and returned to the beakers. They were observed for 30 minutes for running, clonic and tonic fits, loss of postural control and death.
The test drug was deemed effective if it protected the mice from clonic convulsions.

3.2.6.3 Caffeine-induced convulsions

Female AJ albino mice weighing 19-25g were used. They were injected with M. salicifolia extract (100 and 200mg/kg), diazepam (10 and 20mg/kg) 2% gum acacia (control) intraperitoneally and then placed singly in clean 1 litre beakers. 30 minutes later they were injected with caffeine 200mg/kg intraperitoneally and returned to the beakers.

They were observed for half an hour for the occurence of humping fits, clonic or tonic convulsions, loss of postural control or death.

Protection against tonic convulsions and death was the end-point used for determining anticonvulsant activity of the test drugs.

3.2.7 EFFECT OF M. SALICIFOLIA ON PHYSOSTIGMINE LETHALITY

M. salicifolia extract was evaluated for central anticholinergic and antiparkinsonian activity using the method proposed by Nose and Kojima (1970). Mice treated with the extract were challenged with a lethal dose of the acetylcholinesterase inhibitor, physostigmine. Reduction in lethality would indicate central anticholinergic activity.

Although Parkinsonism is primarily due to lesions in dopamine pathways it responds to anticholinergic agents and some like benzhexol and benztropine are in clinical use (Mawdsley and Simpson, 1978, Turner and Richens, 1978). The test seeks to identify other such agents.

The method described by Karasawa et al (1980) was used. Female AJ strain mice weighing 18-30g in groups of ten were injected intraperitoneally with M. salicifolia (200mg/kg) of 2% gum acacia thirty minutes before intraperitoneal injection of physostigmine sulfate (1mg/kg). The number of dead animals was counted after 30 minutes.

3.2.8 EFFECT OF M. SALICIFOLIA EXTRACT ON MOTOR PERFORMANCE

3.2.8.1 The Chimney test

A modification of the method used by Pieri *et al* (1981) to measure impairment of climbing ability in mice was adopted.

Male albino Swiss strain mice were injected with *M. salicifolia* or gum acacia intraperitoneally in groups of 20 animals per dose. At various intervals after drug administration the mice were introduced into one end of a plastic tube of interval diameter 27mm and gently pushed from behind towards the opposite end with a metal rod. The tube was then turned vertically on the table top so that the mouse was placed head down at the bottom end of the tube. The time taken to climb backward up a 20cm distance (identified with a mark) up the tube was measured to the nearest one-tenth second. An animal was deemed to have failed it it took more than 30 seconds to do so.

3.2.8.2 The traction test

The method used by Hirose et al (1981) to measure impairment of climbing ability in mice was used. Male albino Swiss strain mice were injected with M. salicifolia at various doses or the vehicle, 2% gum acacia intraperitoneally. Thirty minutes later the mice were made to hang on a horizontal metal wire 40cm from the bench by grasping and holding with their forepaws. The time taken for the mice to grip the wire with hindpaws was measured to the nearest one-tenth second. The traction response was deemed to be absent if the animal took more than 10 seconds to grip the wire with hindpaws.

3.2.9 INTERACTION OF M. SALICIFOLIA EXTRACT WITH CNS DEPRESSANTS

3.2.9.1 Pentobarbitone sleeping time in mice

Male Swiss albino mice weighing 26-39g were used. *M. salicifolia* (100mg/kg) and the vehicle, 2% gum acacia were injected intraperitoneally into groups of 10 mice. 30 minutes later the animals were injected with pentobarbitone sodium in saline (50mg/kg) intraperitoneally. When the righting reflex was lost, the animals were made to lie with the feet up. Recovery of the righting reflex was deemed to occur when the mice which were placed far apart and undisturbed turned over on their own volition. The interval between the loss and recovery of the righting reflex, the sleeping time, was noted. The experiment was performed between 11.00am and 14.00 hours at a temperature of 20.0°-20.5°C with a background of continuous noise provided by fans.

3.2.9.2 Ethanol sleeping time in mice

The same method used for pentobarbitone sleeping time was used. Ethanol 4g/kg body weight (10ml/kg of a 40% aqueous ethanolic solution) was injected into the mice 30 minutes after *M. salicifolia* or gum acacia.

Ethanol was administered intraperitoneally as oral dosing was observed to produce great variations in duration of sleep.

3.2.9.3 Ether anaesthesia in rats

The method of Bhide (1962) was adopted. Ten pairs of female Sprague-Dawley rats were used, one member of the pair being injected with M. salicifolia extract (100mg/kg) and the other, the control with gum acacia. Each pair was matched as to weight and age. 30 minutes after drug administration each pair of rats was placed in an large inverted funnel (200mm diameter, volume 1.5 litre) and exposed to 2ml diethyl ether for 7 minutes. After 7 minutes the animals were removed from the funnel and the time taken for the recovery of the righting reflex noted.

.2.10 EFFECT OF M. SALICIFOLIA EXTRACT ON BODY TEMPERATURE

.2.10.1 Normal Body temperature

Male Sprague-Dawley rats in groups of 8 were used. The rectal temperature of each rat was determined by inserting the bulb of a children's clinical thermometer lubricated with soft paraffin into the rectum for 60 seconds.

The rats were then injected intraperitoneally with *M. salicifolia* extract (100mg/kg) acetylsalicylic acid (100mg/kg) or the vehicle(2% gum acacia)suspended in sterile normal saline. Rectal temperatures were then measured 2 and 4 hours after drug administration.

3.2.10.2 Brewers yeast-induced fever

Male Sprague - Dawley rats were used. Pyrexia was induced by subcutaneous injection of brewers yeast (Carrano *et al*, 1975, Sofia *et al*, 1977a, Shibata, 1977, Caliari *et at* 1977. Shankaranarayan *et al* 1979).

Baseline rectal temperatures were measured by inserting the bulb of a children's clinical thermometer lubricated with soft paraffin in the rectum of each rat for 60 seconds. The rats were then injected with Brewer's yeast (20% $^{w}/v$; 1ml/100g) in water subcutaneously into the loose connective tissue between the shoulder blades. 20 hours later, the rectal temperature was again measured. The rats in groups of 8 were then given intraperitoneal injections of *M. salicifolia* extract (100mg/kg), acetylsalicylic acid (100mg/kg) and the vehicle, (2% gum acacia), all suspended in sterile normal saline. Rectal temperatures were measured 2 and 4 hours later.

3.3. EFFECTS OF M. SALICIFOLIA EXTRACT ON THE GASTROINTESTINAL TRACT

The effect of M. salicifolia extract on the gastrointestinal tract was investigated both in vivo and in vitro.

3.3.1 Guinea Pig Ileum (in vitro)

The method described by the staff of the Department of Pharmacology, University of Edinburgh (1970a) was used.

A guinea pig fasted overnight was killed with a blow to the head and cutting the throat. A part of the ileum was removed and placed in Tyrode's solution. A 3cm long portion was then cut, threaded at each end and mounted in a 10ml organ bath containing Tyrode's solution at 37°C. The preparation was aerated with carbogen (95% oxygen and 5% carbon dioxide), was left to stabilise in the organ bath for 30 minutes before adding drugs . (Plate 6)

Various amounts of M. salicifolia extract were added to the organ bath. In addition, the effect of adding atropine, hexamethonium and mepyramine maleate respectively 2 minutes before M. salicifolia extract was determined. The effect of acetylcholine on the tissue was also established

3.3.2 Rabbit Jejunum (in vitro)

The rabbit jejunum was set up in a similar way to the guinea pig ileum. The jejunum is that part of the intestine nearest the stomach. Various amounts of *M. salicifolia* extract and acetylcholine were added to the tissue after it stabilised.

3.3.3 In vivo gastrointestinal motility

The method described by Sofia *et al* (1977b) was adopted.

a) Effect in rats

Male Sprague-Dawley rats in groups of seven were deprived of food for 24 hours but water was freely available. Each group was treated with *M. salicifolia* extract orally (30 and 100mg/kg) or intraperitoneally (100mg/kg). Controls received 2% gum acacia by either route.

30 minutes later 7.5% decolorising charcoal suspended in 1% gum acacia was administered orally by gavage. 30 minutes later all the rats were killed with diethyl ether, the abdomen opened and the small intestines removed. The charcoal was visible through the intestinal wall but incisions were made to confirm the position reached by the charcoal meal.

The length of the small intestine and the distance moved by the charcoal meal from the stomach were measured and the ratio computed.

b) Effect in mice

Non-fasted male albino Swiss mice in groups of 8 were used. Each group was injected intraperitoneally with either *M. salicifolia* extract (200mg/kg), codeine (40mg/kg) or the vehicle, (2% gum acacia). After 160 minutes a charcoal meal containing 7.5% decolorising charcoal suspended in 1% gum acacia was administered to each mouse orally by gavage. One hour later the mice were killed with diethyl ether, the abdomen opened and the small intestine removed. The length of the small intestine and the distance moved by the charcoal meal from the stomach were measured and the ratio computed.

3.3.4 Ulcerogenic effects

A modification of the method described by Sofia *et al* (1977b) was used. Male Wistar albino rats in groups of 6 were deprived of food but not water for 24 hours. Each group was treated with either *M. salicifolia* extract (200mg/kg), indomethacin (10mg/kg) or the vehicle, 2% gum acacia.

The animals were then denied access to both food and water for the next four hours. Subsequently, both tap water and mice pellets (Muus Kenya Limited, Thika) were made available. Twenty four hours after administration of the test drugs, all the rats were killed with a blow to the head and cutting the throat. The abdomens were opened and the stomachs removed and immersed in 10% aqueous formalin for 30 minutes to fix the outer wall. The stomachs were then opened along the greater curvature and the contents washed off with a slow running stream of tap water. They were then examined visually for corrosion and with a dissecting microscope (x10) for the presence of gastric ulcers.

3.4. EFFECTS ON THE CARDIOVASCULAR SYSTEM

The cardiovascular effects of M. salicifolia extract were determined on three preparations: blood pressure of the rat, the perfused isolated rabbit heart and the perfused rat hindquarters. The rat hindquarters was used to study the effect of the extract on isolated vascular beds. This preparation was chosen over other isolated vascular bed set-ups e.g. the perfused rat tail (Wade and Beilin, 1970) and the rabbit central ear artery (Burn 1952) because it is easy to set and gives reproducible results over a reasonable length of time.

3.4.1 RAT BLOOD PRESSURE

The method used was that described by the staff of the Department of Pharmacology, University of Edinburgy (1970b).

A male Sprague-Dawley rat weighing 450g was anaesthetised with 0.6ml/100g of a 25% aqueous solution of urethane injected intraperitoneally and fastened to a dissection board. The trachea was then exposed and cannulated.

The femoral vein was exposed, distended by blocking blood flow with a bulldog clip and cannulated with a fine bore plastic cannula. Heparinised saline was injected through the cannula to prevent clotting of the blood.

The common carotid artery, just behind the trachea was located, separated from the vagus, cervical sympathetic nerves and the internal jugular vein and then cannulated. The cannula was connected to a blood pressure transducer (Gould P23 ID, Statham USA) which in turn led to a recorder (Hellige, Freiburg, W. Germany). The animal was kept warm with a dissecting lamp. (Plate 7)

Drugs were injected intravenously through the femoral cannula. To avoid blocking capillaries, the supernatant of the M. salicifolia extract suspension was rendered particle free by passing through a membrane filter (Millipore, pore size 0.45μm). In addition to being injected alone, M. salicifolia extract was also injected after atropine sulfate (240mg, the dose found to abolish the depressor effect of acetylcholine 0.25mg).

3.4.2 ISOLATED PERFUSED RABBIT HEART

The method described by the Staff of the Department of Pharmacology, University of Edinburgh (1970a) was used.

A large rabbit was killed, the chest opened and the heart with a length of aorta attached rapidly removed and placed in Locke solution in a petri-dish. Blood was removed by squeezing a few times and the heart was mounted on the Langendorff's apparaturs (Plate 8) and perfused through the aorta. The perfusion fluid was oxygenated Locke solution at 37^0C. A small heart clip was attached to the base of the heart. A thread from the clip led to an isometric transducer (Washington Type D) which was wired to an Oscillograph (Washington 400 MD 2C) on which the contractions were recorded.

Drugs were injected through the rubber cap above the cannula leading to the aorta. M. salicifolia extract was rendered particle free by passing the supernatant through a membrane filter (Millipore Type HA, pore size 0.45μm).

3.4.3 PERFUSED RAT HINDQUARTERS

The method used was a modification of that of Maitai (1973)

A male adult Sprague-Dawley rat was anaesthetised with 0.6ml/100g of a 25% aqueous solution of urethane. The abdomen was opened and the abdominal aorta cannulated. The rat was then cut in two and the upper half discarded. The hindquarters were then connected to a Langendorff apparatus (Plate 8) and perfused with Tyrode solution containing 2% w/v gelatin at 37°C and aerated with carbogen (95% oxygen and 5% carbon dioxide).

The perfusion pressure was recorded on a Hellige BP recorder (Freiburg, Germany) by connecting a pressure transducer (Gould P23 ID Statham, USA) to the perfusion fluid just behind the cannula. The perfusate was collected in a measuring cylinder and the perfusion rate determined at one minute intervals.

Drugs were injected through the rubber cap above the cannula. M. salicifolia extract was rendered particle free by passing through a Millipore membrane filter. The suitability of the preparation was tested by determining the effect of adrenaline 10μg and acetylcholine 2μg on the perfusion pressure.

2.5 EFFECT OF M. SALICIFOLIA EXTRACT ON OTHER ORGAN SYSTEMS

2.5.1. Diuresis In The Rat

Male albino Sprague-Dawley rats weighing 400-500g

were deprived of food but not water for 24 hours. On the test day each rat was hydrated with 25ml warm distilled water. The test drugs were then administered either orally or by intraperitoneal injection. The rats were then placed in pairs in metabolic cages without food or water and the urine produced was collected over a 6 hour period under liquid paraffin to stop evaporation.

The test drugs were M. salicifolia extract (100 and 200mg/kg), frusemide (20mg/kg) and 2% gum acacia for the controls.

3.5.2 ANTIINFLAMATORY ACTIVITY OF M. SALICIFOLIA EXTRACT - CARRAGEENAN PAW EDEMA

The antiinflammatory activity of M. salicifolia extract was assessed using the Carrageenan rat paw edema method of Winter et al (1962) as modified by Shibata (1977).

Adult male Wistar rats in groups of 6 weighing 200-270g were deprived of food but not water for 24 hours. Each group was administered with either M. salicifolia extract (200mg/kg) or indomethacin (10mg/kg) orally by gavage. Controls were treated with the vehicle, 2% gum acacia solution.

One hour later the volume of the right hind foot was measured by mercury displacement in a locally designed plethysmometer (Plate 7). This consists of a rubber tubing filled with mercury connecting on one side calibrated glass capillary tube and on the other a hollow plastic cyclinder partially filled with mercury and with a bore large enough to fit a rat's foot. The foot was immersed in the mercury in the plastic tube up to the level of lateral malleolus and the volume of the displaced mercury measured in the capillary tube. Immediately after measuring the foot volume 0.1ml of a 1% carrageenan solution in sterile normal saline was injected into the plantar surface of the paw, and the animal returned to the cage.

Three hours later the volume of the same foot was again measured. The difference between the two volumes was designated the edema volume.

3.5.3 LOCAL ANAESTHETIC ACTIVITY - THE GUINEA PIG WHEAL

Local anaesthetics are drugs that block nerve conduction when applied locally in appropriate concentrations. Nerve blockade is indicated when stimulating a motor nerve fails to produce a muscle contraction, or a sensory nerve fails to produce a reflex response.

The guinea pig wheal was adopted to test M. salicifolia extract for local anaesthetic activity. An active drug blocks the twitching response to stimulation of sensory nerves by pricking the animal.

Four male coloured guinea pigs were used.

Twenty four hours before use, the hair on the lower back was clipped with scissors and the depilatory cream, Veet-0 was then used to shave them clean. Four sites near the midline (the most sensitive area) were chosen on each animal for injecting the drugs. Procaine hydrochloride (3mg/ml), saline and a particle free solution of M. salicifolia extract (10mg/ml) were injected intradermally at 0.25ml volume to form wheals. Each wheal was outlined with felt pen. Each drug was allocated at least one injection site in each animal.

The wheal was then pricked lightly with a needle six times every 5 minutes for half an hour. Six twitches, one for each prick indicated no anaesthesia, while lack of twitches indicated total anaesthesia. The total number of twitches for the wheals of each drug were obtained and the means compared.

3.5.4 THE NEUROMUSCULAR JUNCTION - RAT PHRENIC NERVE PREPARATION

This preparation contains a neuromuscular junction and is used to study the effect of drugs on the contractions of the muscles of the diaphragm after electrical stimulation of the phrenic nerve. The neurotransmitter at the neuromuscular junction is acetylcholine.

A large male rat was killed with a blow to the head and bled through the carotid arteries. The skin and pectoral muscles of the left side were removed, the thorax was opened and the ribs also removed. The phrenic nerve was located, ligated on the cranial side and freed upto the diaphragm. Two converging cuts were then made through the arcus costarum towards the tendinous part of the diaphragm to give a hemi-diaphragm.

The nerve was then cut near the thymus and the preparation transfered into Kreb's solution in a petri-dish. The costral margin of the diaphragm was attached to the electrode holder and the tendinous end to a isometric transducer (Washington Type D) which was wired to an oscillograph (Washington 400 MD 2C).

The nerve was wound lightly over the electrode and the preparation mounted in a 40ml organ bath containing Krebs - Henseleit solution at 37°C and aerated with carbogen (Plate 6). The nerve was stimulated at 12Hz with a rectangular wave pulse of 0.5 msec duration.

M. salicifolia extract was added to the organ bath and a 10 minute cycle used between drug additions.

3.5.5 EFFECT OF M. SALICIFOLIA EXTRACT ON THE GUINEA PIG TRACHEA

The method of Jamieson (1962) was used. A large guinea pig was killed with a blow to the head and the throat cut very near the head. The trachea was dissected out and placed in Kreb's solution and extraneous tissue removed. It was then attached to a U-shaped capillary tube containing Kreb's solution such that the lumen of the trachea was filled with the solution (Figure 2).

The preparation was mounted in a 10ml organ bath containing Krebs - Henseleit solution at 37°C aerated with carbogen.

After stabilising for 20 minutes, M. salicifolia extract was added into the organ bath. Contractions or relaxations of the trachea cause rises and falls respectively of the level of solution in the calibrated fine bore capillary tube attached to it.

3.5.6 EFFECT OF M. SALICIFOLIA EXTRACT ON THE RAT UTERUS

The method of the staff of the Department of Pharmacology, University of Edinburgh (1970a) was used.

A young virgin female rat weighing approximately 150g was injected subcutaneously with 0.5ml of a saturated solution of stilboestrol 24 hours before use. This was to bring the rat to oestrous and hence increase the sensitivity of the uterus to drugs.

The animal was killed and the abdomen opened to expose the uterine horns which were then removed and placed in a dish containing De Jalon's solution. The two horns were freed of extraneous tissue, separated at the bifurcation and then opened longitudinally to give a sheet of muscle. This was mounted in De Jalon's solution at 30^{0}C and aerated with carbogen. (Plate 6) The preparation was allowed to stabilise for 30 minutes before adding drugs.

Varying doses of M. salicifolia extract, acetylcholine and atropine were added to the organ bath.

3.6 ACUTE TOXICITY

Male Swiss mice weighing 20-30g were injected intraperitoneally with *M. salicifolia* extract in logarithmically spaced doses ranging from 100mg/kg to 500mg/kg body weight. Ten mice were used for each dose and the number of deaths was recorded after 24 hours. The dose range used was chosen after preliminary tests with groups of 2 mice had shown that at 100mg/kg no deaths occured and that at 500mg/kg all the mice died.

The survivors were kept for one more day to enable the detection of delayed toxicity.

3.7 STATISTICAL ANALYSIS OF THE DATA

The statistical method used was that which was found most appropriate for the particular set of data. For instance, when the means between groups were close together but the variances were different the analysis of variance (ANOVA) or the variance ratio test were used. Conversely, when the group means were much different the student t test was used. A Memory 7000 microcomputer and a Casio programmable calculator were used to process the data.

(i) Data from open field apparatus.

Because there was a lot of intra-group variation (that is, great differences in measurements from mice in the same group) logarithmic transformation was performed on all measurements. This avoids a single large measurement skewing the overall result upwards. Subsequently, the data was analysed by analysis of variance (ANOVA) and Newman - Keul's test of ascending grouped means.

(ii) Data on hypnosis potentiation by ethanol and pentobarbitone.

The logarithmic rank method was used to estimate median sleeping time for each group. These were then compared using chi - square test with one degree of freedom.

(iii) Data on duration of ether anaesthesia.

Since the pairs of rats used were matched as to weight, age and sex, the method of paired differences was used. The student t test was then used.

(iv) The student t test was used on data from carrageenan - paw edema, gut motility, diuresis, and the experiments on the effects on body temperature.

(v) Data from hot plate experiment.

The method of paired differences was used, since it was the reaction time of the same mice that was being compared pre - and post-drug. The variance ratio test was then used to compare the differences.

(vi) Data from tail pressure test.

Total analgesia scores were compared with the standardized normal deviate method, while the median reaction threshold was calculated with the logarithmic rank method.

(vii) Data from behavioural despair test.

The standardized normal deviate method was used.

(viii) Acute toxicity

The median lethal dose (LD_{50}) was calculated by the graphical method of Litchfield and Wilcoxon (1949). The logarithm of the doses was plotted against the percentage of mice dying in 24 hours, on semi-logarithmic paper. The median lethal dose (LD_{50}) was read directly from the graph. The standard error of the LD_{50} was calculated from

$$SE = \frac{LD_{84} - LD_{16}}{\sqrt{2N}}$$

where LD_{84} - Dose killing 84% of the mice

LD_{16} - Dose killing 16% of the mice

N - no of mice between the intervals LD_{84} and LD_{16}

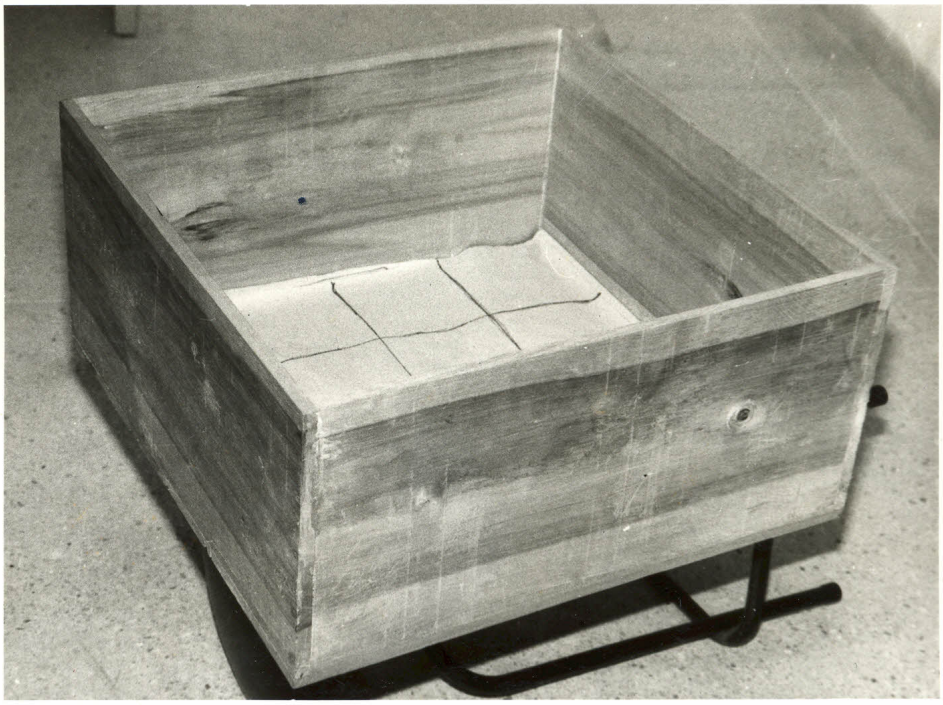


Plate 3 - The open field apparatus

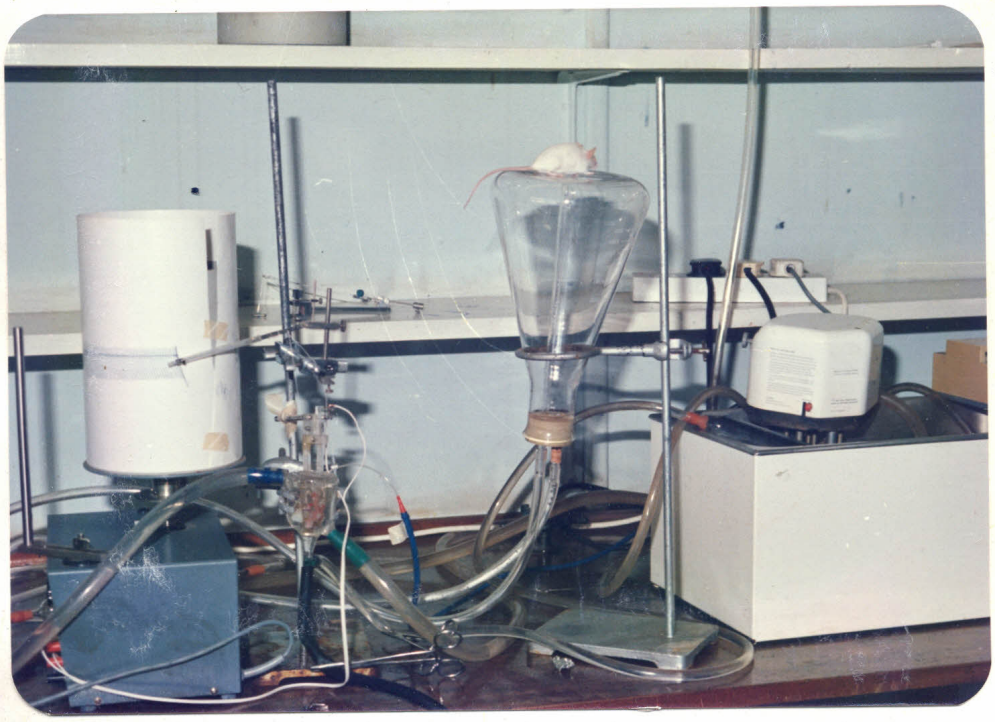


Plate 4 - A mouse on the hot plate - an inverted glass flask.

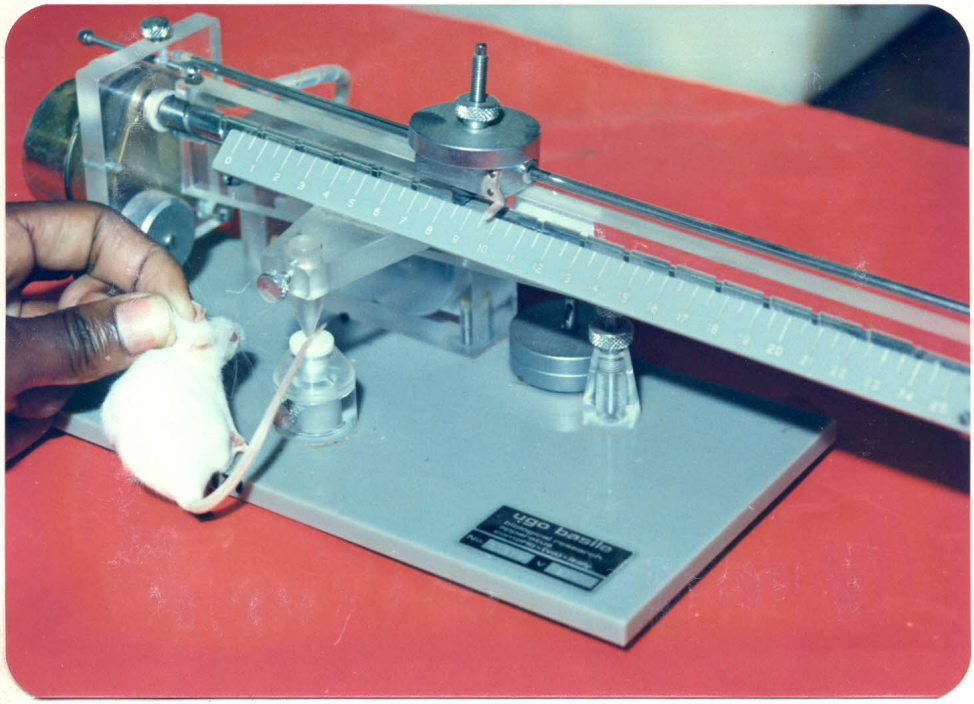


Plate 5 - The Analgesy - Meter [R] - pressure being exerted on a mouse's tail.

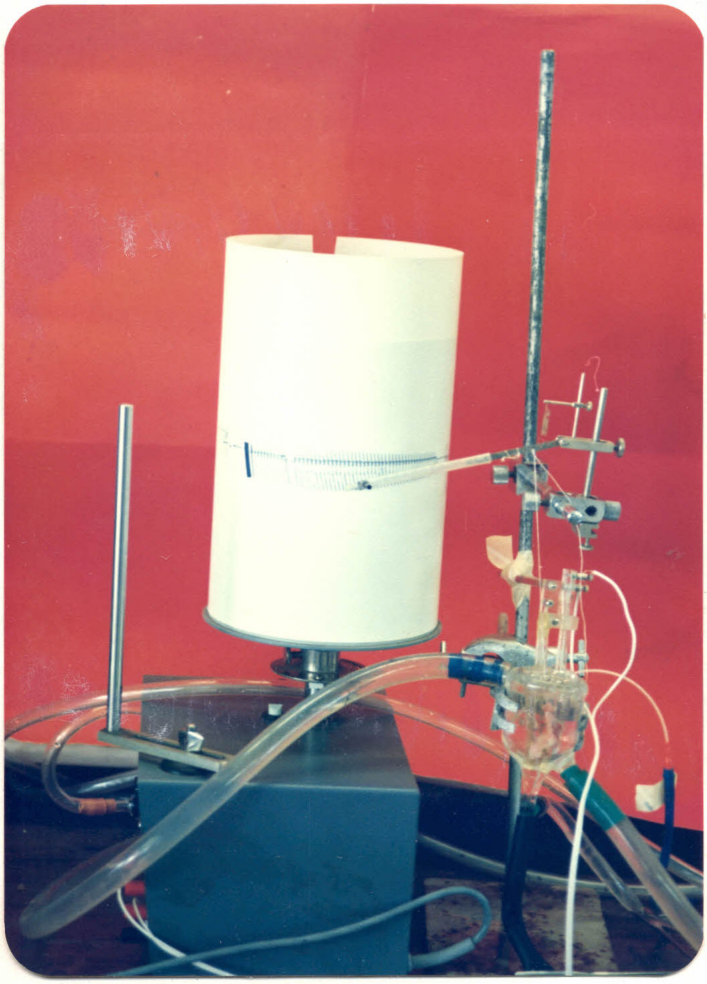


Plate 6 - Apparatus for in vitro study of drugs on guinea-pig ileum, rat phrenic - nerve Diaphragm, rat uterus and rabbit jejunum.

Plate 7 - The apparatus for measuring blood presure of the anaesthetised rat.

Plate 8 - Langendorff's apparatus - used for isolated perfused rabbit heart and perfused rat hindquarters experiments.

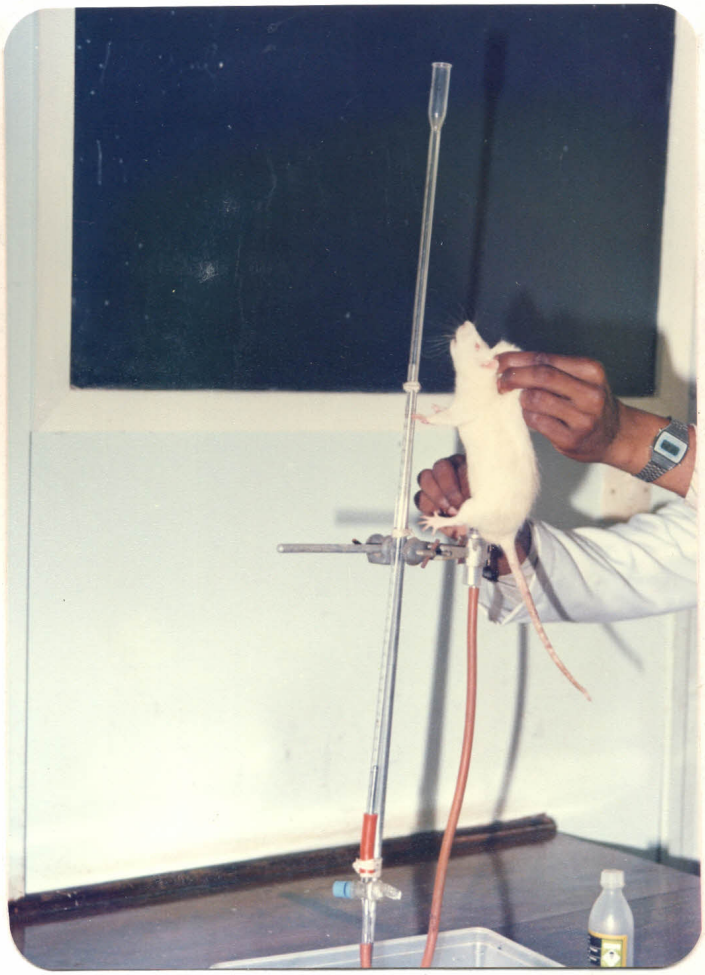
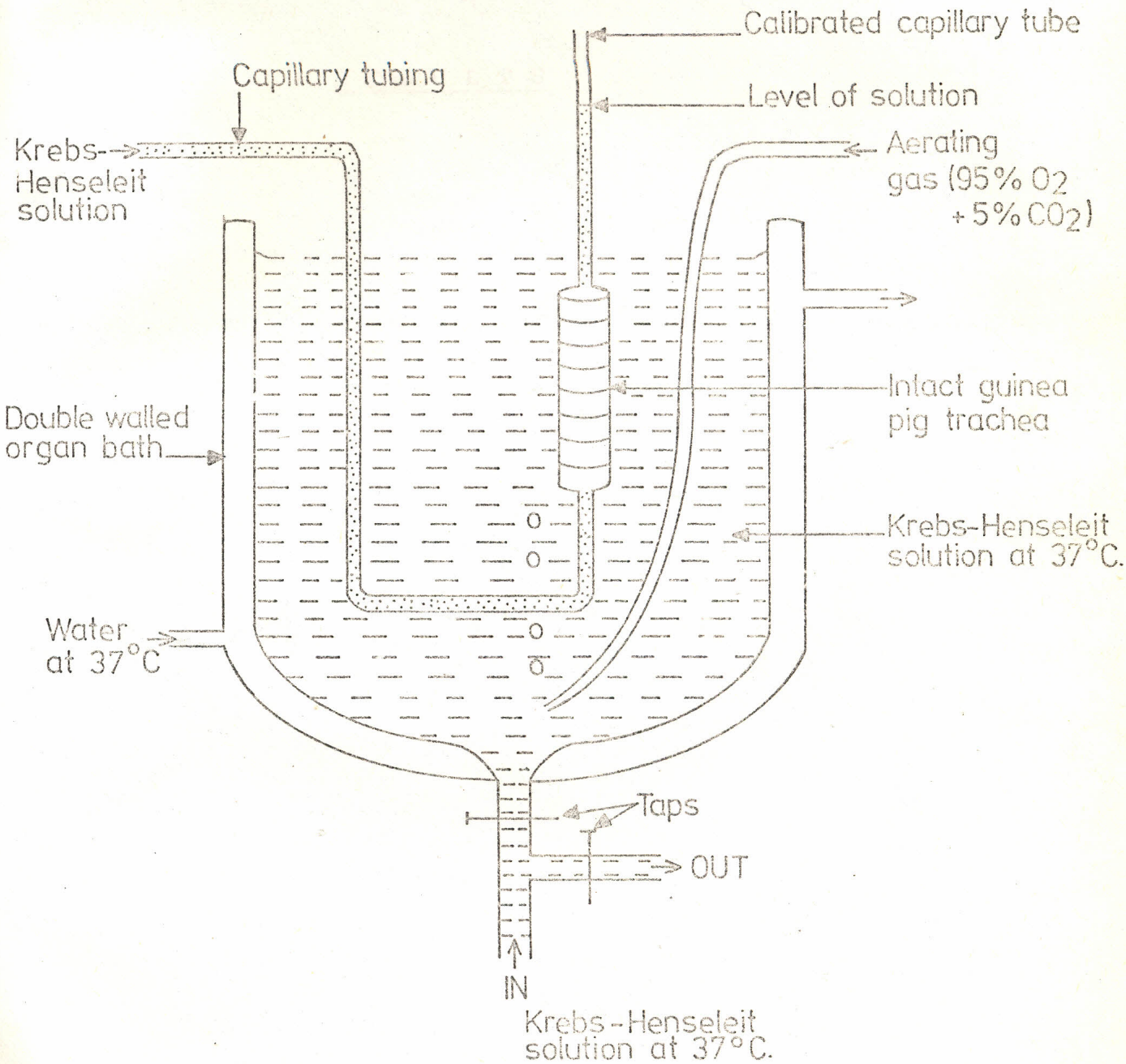


Plate 9 - Measuring the paw volume of a rat with a plethysmomemeter.

FIG 2. APPARATUS FOR INVESTIGATING EFFECTS ON GUINEA PIG TRACHEA



R E S U L T S

Chapter four

RESULTS OF PHYTOCHEMICAL INVESTIGATIONS

4.1 Phytochemical screening

Results are summarised in Table 1.

Tests for alkaloids, anthraquinones, saponins, flavonoids, steroids and cardiac glycosides were negative. Tests for phenolic substances and tannins were positive.

4.2 Thin layer chromatography (TLC)

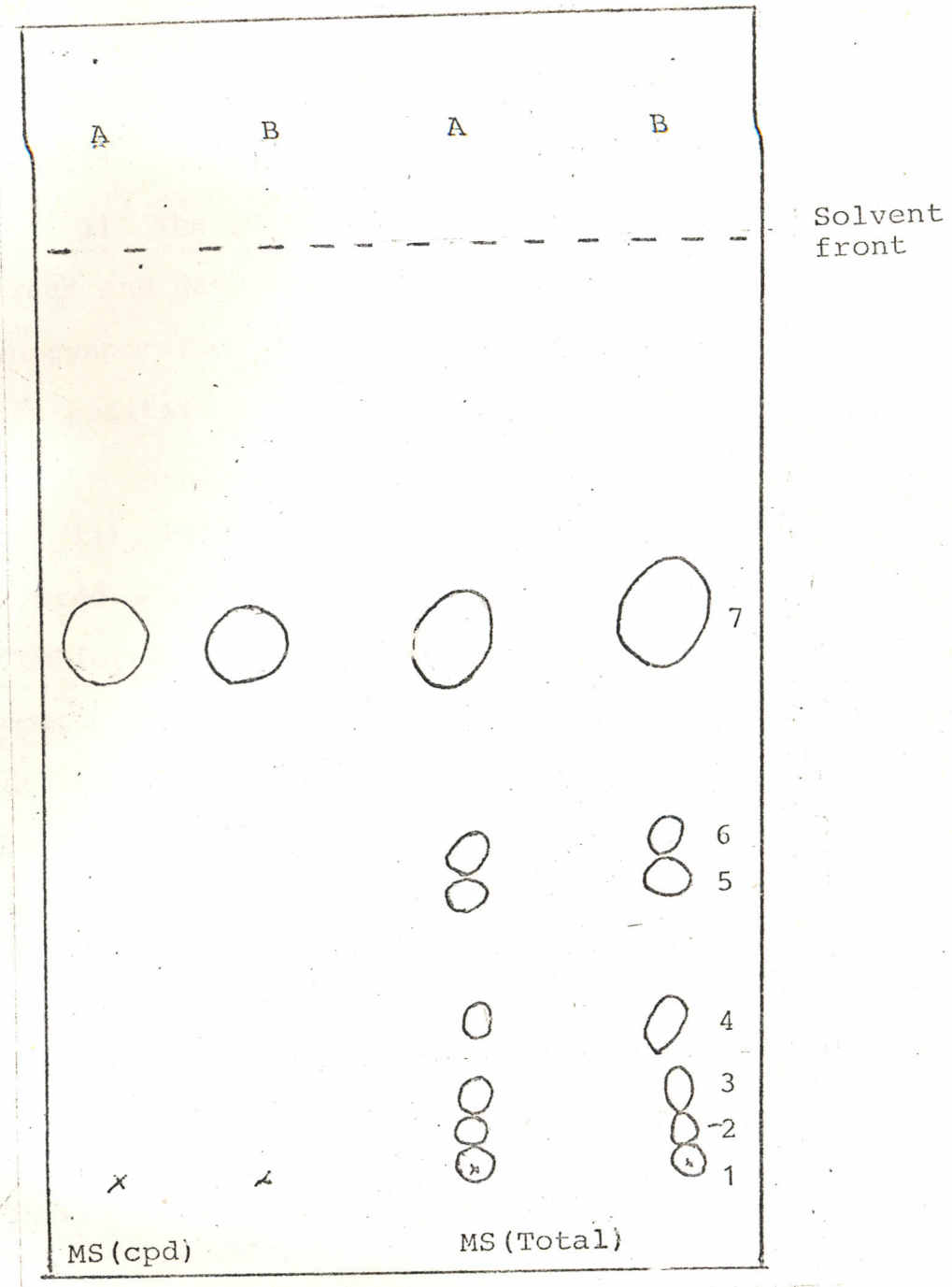
When TLC's of *M. salicifolia* extract were visualized under uv light six spots which fluoresce a bright purple are seen. In addition a yellow spot was seen in visible light. When the plate is sprayed with Ferric chloride - potassium ferricyanide aqueous solution, the six uv fluorescent spots took a deep blue colour indicating that they were phenolic substances. On spraying with Folin-Ciocalteau reagent six blue spots which corresponded to the ones responsive to ferric chloride - potassium ferricyanide spray could be seen.

Fig 3 is a typical chromatogram of the extract and of the compound isolated from the extract. This compound (spot 7, Rf = 0.6) appeared to be the main component.

Some other spots emerged on spraying the TLC plate with 1% anisaldehyde spray (in concentrated sulphuric acid/ acetic acid (2:100)) indicating that the extract contains some terpenoids (Waldi 1965).

TABLE 1 : RESULTS OF PHYTOCHEMICAL SCREENING

TEST FOR	RESULT
Alkaloids	Absent
Anthraquinones	Absent
Saponins	Absent
Flavonoids	Absent
Phenolics	Present
Tannins	Present
Steroids	Absent
Cardiac Glycosides	Absent



A - Under uv light

B - After Folin spray

2 - Yellow in visible light, non-fluorescing.

Adsorbent : Silica gel G-UV_{254}

Solvent system: Isopropanol; methanol: Ammonia.
85 15 10

Fig: 3: Typical TLC of M. salicifolia extract

4.3 Column Chromatography

i) The petroleum ether eluate from the column was clear and gave a single uv fluorescent component on TLC, on evaporation a white semi-solid was obtained. It was positive on tests for phenols.

ii) Petroleum ether - chloroform (3:1) initially eluted a clear solution and later a light yellow solution. The former contains in addition to two phenolic compounds a non-fluorescing triterpene which was detected with anisaldehyde spray. The yellow eluate contained a yellow spot in addition to the other three spots.

iii) All other eluates, viz petroleum ether - chloroform, mixtures of chloroform and methanol, and methanol eluted what turned out to be mixtures of compounds when examined on TLC.

The petroleum ether, petroleum ether - chloroform and chloroform eluates all contained the fluorescent phenolic compound nearest to the solvent front.

4.4 Quantitation of M. salicifolia extract

The scan on ultraviolet and visible light of the methanolic solution of the root extract showed only one peak.

This was at 211nm. This is shown on Fig 4 . The small peaks around 190nm are due to methanol.

The absorbances of different concentrations of the extract are shown on Table 2 . When the absorbance was plotted against concentration, the plot were found to be linear at these concentrations.

To establish the $E^{1\%}_{1cm}$ of the extract, that is the absorbance of 1% solution through a pathlength of 1cm. Taking for instance, absorbance of

10µg/ml extract is 0.791,

10µg/ml = 0.001% $^{w}/_{v}$.

then, in a 1 cm long cell,

$$\text{Absorbance of 1\% solution} = \frac{\text{Absorbance}}{\text{Concentration}}$$

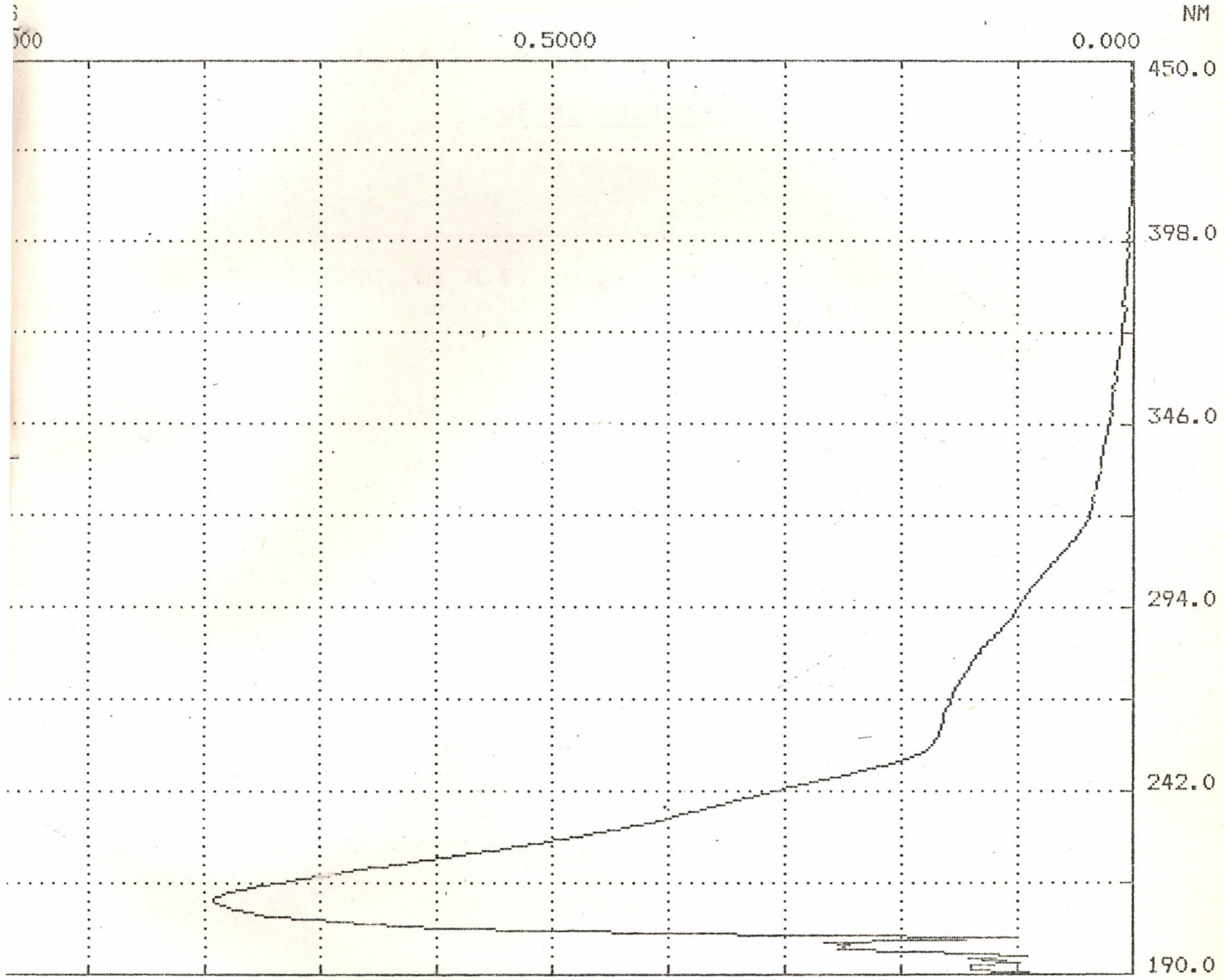
$$= \frac{0.791}{0.001}$$

$$= 791$$

This indicates that the extract is now quantifiable, with a known concentration corresponding to a particular absorbance.

BECKMAN DU-6 SPECTROPHOTOMETER

REPLOT
ABSORBANCE



SCAN SPEED: 300 NM/MIN

SOURCES: VIS/UV

SLIT: _____ NM

DATE: 4|3

OPERATOR: K N

SAMPLE: MS 10 µg/ml

REFERENCE: Methanol

PEAK PICK

λ	ABS	λ	ABS
211.0	0.791		
197.0	0.254		
191.0	0.140		

FIG. 4 : UV Spectrum of M. Salicifolia extract

TABLE 2 : UV Absorbances of various concentrations of M. salicifolia extract at 211nm

CONC. OF M.S. µg/ml	Absorbance at 211nm
5	0.398
10	0.791
15	1.112
20	1.428
25	1.799

4.5 Partial characterisation of the compound isolated from M. salicifolia roots.

The white semi-solid eluted by petroleum ether from the column was found to be one component when run in TLC on several solvent systems. It was therefore considered to be a single compound and denoted MS(A). The Rf values on these solvent systems are shown on Table 3 . Rf is the ratio of the distance moved by the compound from the base line to the distance moved by the solvent front.

The compound was soluble in petroleum ether but insoluble in ethanol and methanol. It was crystallized in methanol. From the intensity of uv fluorescence and the blue spots on spraying with Folin and ferric chloride - potassium ferricyanide sprays it was observed to be the main component (Fig 3). The quantity obtained was however too little for pharmacological testing to prove if it was the active principle.

The infra-red spectrum of this compound is shown on Fig. 5 . The absorptions observed are interpreted as follows;

i) Sharp C-H stretching at 3000 cm^{-1} - alkane

ii) Shoulder at $3080cm^{-1}$ - Aromatic centre

iii) Sharp C=O stretching vibrations at $1750cm^{-1}$ - saturated cyclic chromophone, probably five-membered.

iv) c - c stretching vibrations at $=1600cm^{-1}$ - aromatic centre

v) c - c stretching at $1610cm^{-1}$ - alkene.

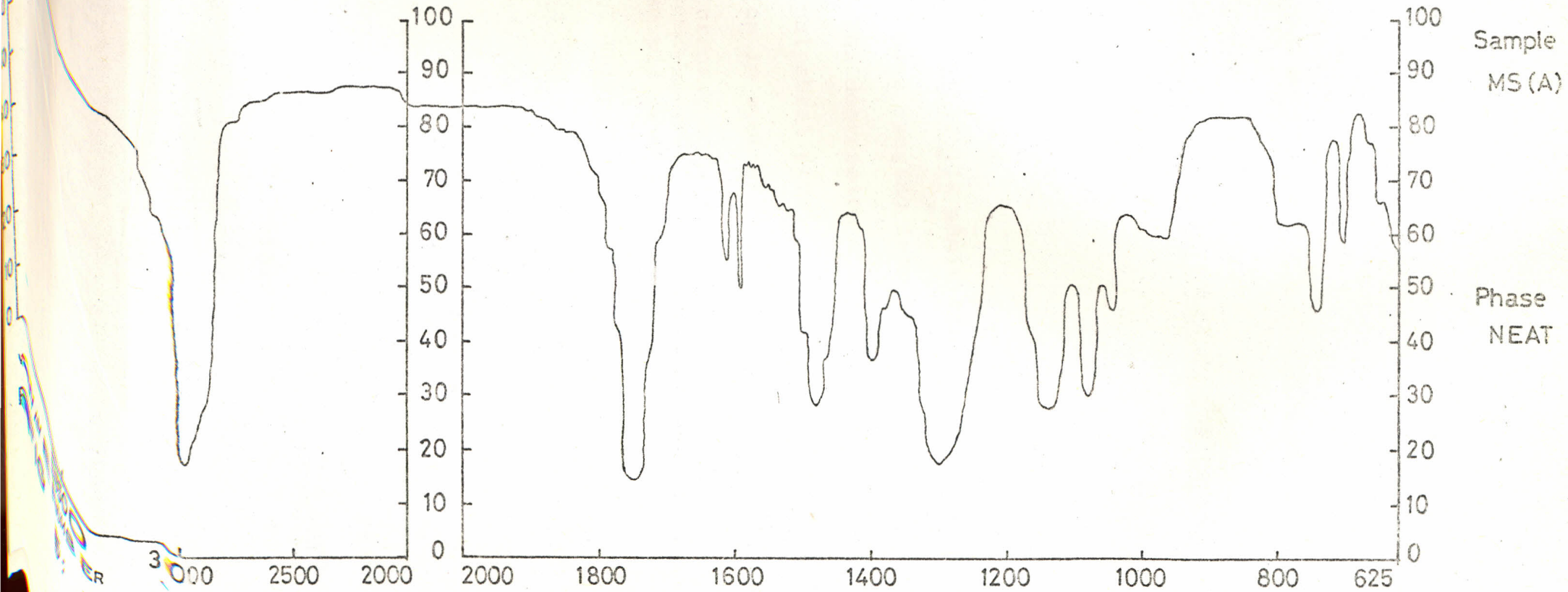
With an aromatic nucleus, a cyclic carbonyl group and the phenolic group observed on TLC, it is most likely that this compound was a phenylpropanoid probably a coumarin derivative.

The ultraviolet (uv) spectrum of MS (A), the isolated compound was a broad band instead of a peak and was difficult to interpret.

TABLE 3 : Rf (x100) VALUES OF THE COMPOUND ISOLATED FROM M. SALICIFOLIA ROOTS, IN SEVERAL SOLVENT SYSTEMS

SOLVENT SYSTEM	Rf x100
1. Isopropanol - methanol - Ammonia 85 15 10	63
2. Methanol - chloroform - Ammonia 85 13 -2	75
3. Butanol - Acetic Acid - Water 4 1 5	75
4. Chloroform	61
5. Hexane : Diethyl ether 50 50	79

On silica gel plates.



INFRA RED SPECTRUM OF MS(A)

Chapter Five

RESULTS OF PHARMACOLOGICAL INVESTIGATION

5.1 EFFECTS OF M. SALICIFOLIA ON THE CENTRAL NERVOUS SYSTEM

5.1.1 Gross behaviour

M. salicifolia administered intraperitoneally in mice in doses ranging from 10 - 400mg/kg produced dose related CNS depression characterised by sedation, slight palpebral ptosis, ataxia, catalepsy, analgesia, reduction in spontaneous and motor activity, indifference to disturbance and reduction in respiratory rate. There was also piloerection and vasodilation. At the highest doses M. salicifolia extract produced excitation, writhing and stereotyped grooming. Sleep was not produced.

In rats, the effects of M. salicifolia extract were more pronounced than in mice. Writhing was observed within 5 minutes, followed by profound sedation, muscle relaxation, ataxia, catalepsy and almost total palpebral ptosis. There was however no loss of the righting reflex.

5.1.2 Open field activity

Results are shown on Tables 4-7 and Figs 6-8. M. salicifolia extract in doses of 10, 25, 50 and 100mg/kg body weight injected intraperitoneally reduced the number of squares transversed (a measure of horizontal movement), the number of rearings (a measure of vertical movement) and the number of grooming bouts in mice compared with controls (Table 4 and 4-5 Fig 6, 7, 8).

There was no significant difference between the effect of the various doses on any of the components of open field behaviour. The number of urinations and defaecations in both the control and test groups was too few for statistical analysis (Table 7). By comparison amitriptyline (20mg/kg) and haloperidol (2mg/kg) reduced the number of squares transversed but amitriptyline (10mg/kg) did not (Table 6, and Fig 7). Amitriptyline (20mg/kg) reduced the number of rearings but amitriptyline (10mg/kg) and haloperidol (2mg/kg) did not (Fig 8). Diazepam (10mg/kg) despite causing noticeable ataxia, increased the number of squares trasversed but did not change the number of rearings (Fig 7 and 8).

Amphetamine (3.75mg/kg) increased both the number of squares transversed and the number of rearings. (Table 6).

All the test drugs decreased the number of grooming bouts but the decrease due to haloperidol (2mg/kg) and amphetamine (3.75mg/kg) was not statistically significant.

ABBREVIATIONS USED IN THE TABLES

AMI - Amitriptyline

MS - *M. salicifolia* extract

S - Number of squares transversed

R - Number of rearings

G - Number of grooming bouts

DZP - Diazepam

HAL - Haloperidol

AMP - Amphetamine

N - Number of animals per group

C - Controls.

TABLE 4: OPEN FIELD BEHAVIOUR OF MICE 30 MINUTES AFTER DRUG TREATMENT (1)

DRUG	CONTROL			AMI. 10MG/KG			AMI. 20MG/KG			MS 50MG/KG			MS 100MG/KG		
MOUSE	S	R	G	S	R	G	S	R	G	S	R	G	S	R	G
1	88	29	3	49	17	0	20	6	2	5	0	2	15	0	0
2	87	27	4	53	11	0	33	4	0	10	0	0	1	2	0
3	81	18	6	23	5	0	45	0	0	37	4	0	5	0	0
4	46	23	4	36	2	0	64	17	0	10	0	0	6	1	3
5	30	2	13	52	12	0	27	0	0	2	0	0	28	3	0
6	59	25	0	80	9	4	29	9	0	37	3	5	2	0	1
7	64	15	11	56	20	0	21	0	0	8	0	10	40	4	5
8	61	14	0	33	22	4	48	0	0	5	0	0	23	5	6
9	53	11	0	75	8	3	53	0	0	17	2	6	27	0	0
10	78	11	0	45	3	11	6	0	2	7	0	0	15	0	0
11	38	23	13	44	14	4	32	1	2	3	0	11	15	2	3
12	27	1	8	90	22	3	37	0	0	6	0	3	20	0	7
13	30	9	9	76	21	9	18	0	0	20	1	9	4	1	0
14	21	1	16	32	4	0	23	4	0	19	1	1	87	34	0
15	55	10	17	23	2	0	47	0	0	26	1	8	3	0	0
MEAN	53.7	14.6	6.9	50.5	11.5	2.5	33.5	2.1	0.4	14.5	0.8	3.7	19.4	3.3	1.7
						**		**	**	*	**	**	**	**	**

* $p < 0.05$

** $p < 0.01$

TABLE 5: OPEN-FIELD BEHAVIOUR OF MICE 30 MINUTES AFTER DRUG TREATMENT (11)

DRUG	CONTROL			M. SALICIFOLIA 10MG/KG			M. SALICIFOLIA 25MG/KG		
MOUSE	S	R	G	S	R	G	S	R	G
1	34	16	3	3	0	0	2	0	0
2	42	14	6	2	1	0	13	0	0
3	53	16	5	21	1	4	10	0	8
4	19	6	5	11	2	0	49	14	2
5	31	9	2	40	10	4	14	1	0
6	43	14	5	17	1	0	5	1	0
7	21	1	17	6	1	4	1	0	0
8	45	20	9	8	0	0	28	1	2
9	91	21	1	14	0	1	28	21	4
MEAN	42.4	13	5.9	13.6	1.78	1.44	16.7	4.33	1.78
				*	*	*	*	*	*

* $p < 0.05$

S = number of squares
R = " " rearings
G = " " groomings

TABLE 6: OPEN FIELD ACTIVITY OF MICE 30 MINUTES AFTER DRUG TREATMENT (111)

	CONTROL			DZP 10MG/KG			AMI 10MG/KG			HAL 2MG/KG			AMP 3.75MG/KG		
MOUSE	S	R	G	S	R	G	S	R	G	S	R	G	S	R	G
1	38	11	9	12	6	0	2	5	4	21	17	2	14	23	2
2	26	7	2	64	6	1	13	5	5	13	0	8	93	23	1
3	28	10	3	98	27	1	6	0	2	38	16	5	90	23	0
4	26	5	7	92	7	3	31	12	1	10	1	3	24	5	5
5	37	6	6	33	3	8	38	2	6	27	11	13	27	1	14
6	48	14	2	87	28	1	10	1	3	7	1	5	47	7	7
7	47	13	9	37	8	0	90	8	4	37	7	2	10	2	7
8	35	15	21	25	0	7	10	3	6	24	16	3	163	34	2
MEAN	35.6	10.1	7.4	56	10.6	2.6	25	4.5	3.9	22.1	8.6	5.1	58.5	14.8	4.8
				*									*		

* $p < 0.05$

DZP = Diazepam
AMI = Amitriptyline
HAL = Haloperidol
AMP = Amphetamine
S = Number of Squares
R = " " rearings
G = " " groomings

TABLE 7: OPEN FIELD BEHAVIOUR IN MICE 30 MINUTES AFTER DRUGS - NUMBER OF URINATIONS AND DEFAECATIONS

DRUG	N	TOTAL DEFAECATION	TOTAL URINATIONS
CONTROL	25	8	0
MS 10MG/KG	9	2	0
MS 25MG/KG	9	1	1
MS 50MG/KG	15	0	1
MS 100MG/KG	15	1	3
AMPHETAMINE 3.75MG/KG	9	5	1
AMITRIPTYLINE	15	0	1
AMITRIPTYLINE	15	0	0

MS = M. salicifolia extract

N = Number of mice

FIG.6: NUMBER OF SQUARES TRANSVERSED AS PERCENTAGE OF CONTROL (MEAN). (OPEN FIELD BEHAVIOR)

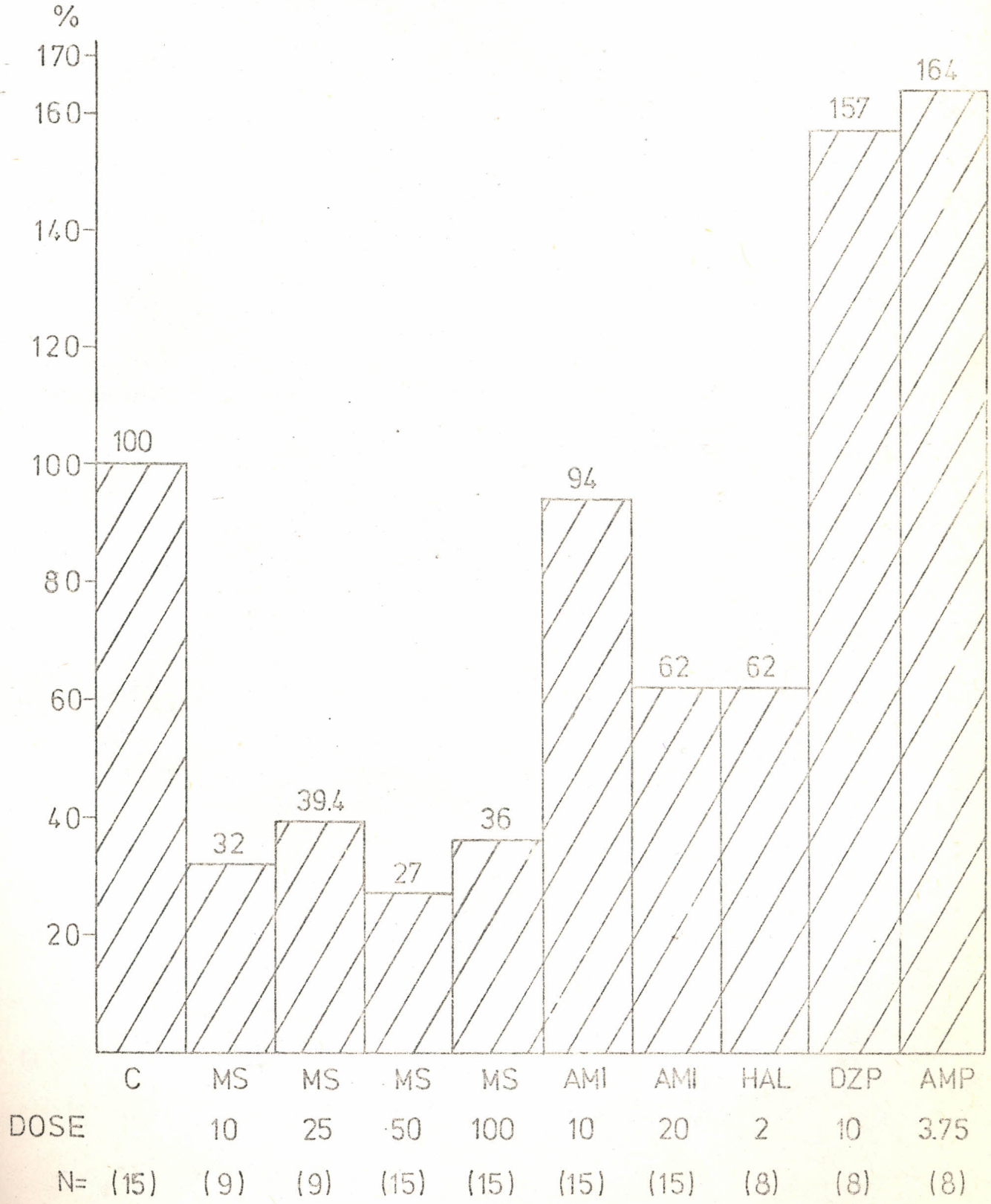


FIG.7: NUMBER OF REARINGS AS PERCENTAGE OF CONTROL
(OPEN FIELD BEHAVIOR)

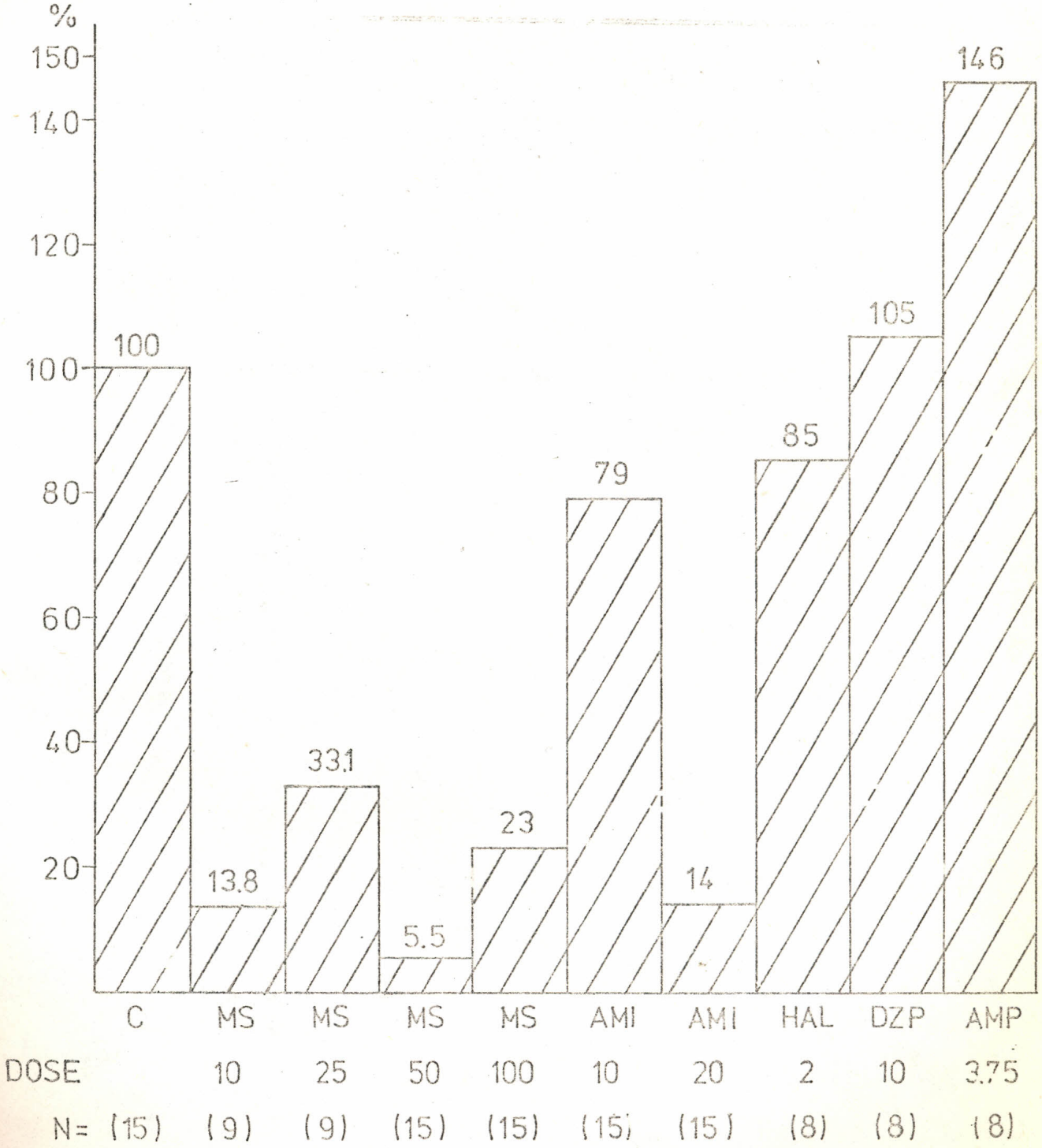
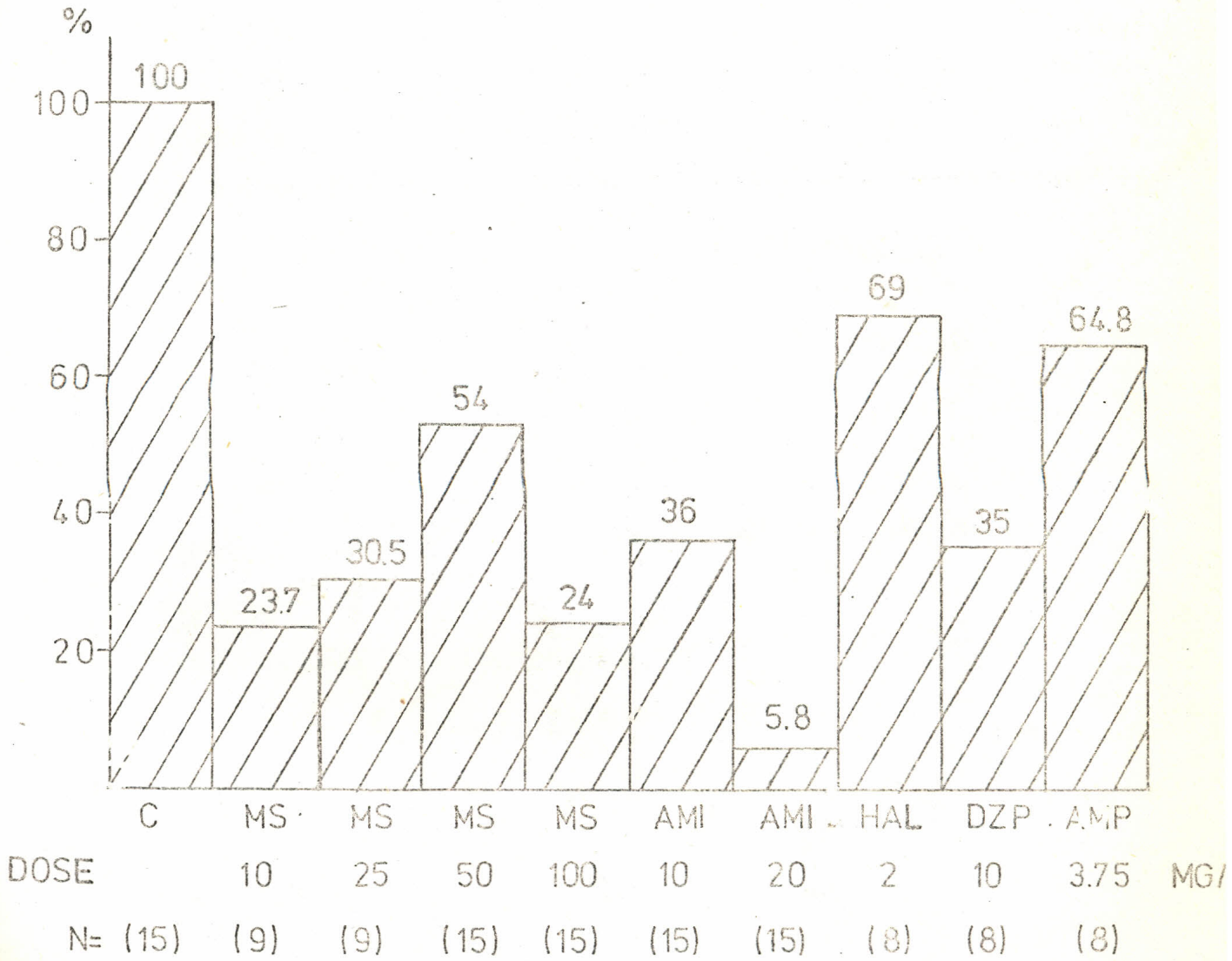


FIG.8: NUMBER OF GROOMINGS AS PERCENTAGE OF CONTROL (MEAN), (OPEN FIELD BEHAVIOR)



5.1.3 TESTS FOR NEUROLEPTIC ACTIVITY

5.1.3.1. Amphetamine hypermotility

Results are shown on Tables 8-11

Administration of dexamphetamine 3.75mg/kg i.p increased the open field activity of mice on all three measures - squares transversed, rearings and grooming. (Table 8) This increase was more pronounced when the open field activity was measured 60 minutes instead of 30 minutes after dexamphetamine administration.

Pretreatment of mice with M. salicifolia 10 and 25mg/kg ip 30 minutes before amphetamine reduced this amphetamine hyperactivity in a dose dependent manner. (Table 9 and 10). M. salicifolia at 10mg/kg did not significantly affect the horizontal movement (squares transversed) but it reduced the number of rearings and groomings. M. salicifolia at 25mg/kg reduced all the characteristics: squares transversed, rearings and groomings. This reduction is more evident when the open field activity was measured 30 minutes rather than 60 minutes after d-amphetamine injection.

TABLE 8: OPEN FIELD BEHAVIOUR IN MICE - BEFORE, 30 AND 60 MINUTES AFTER AMPHETAMINE (3.75MG/KG) ADMINSTRATION

	BEFORE AMPHETAMINE			30 MIN AFTER AMPHETAMINE			60 MIN AFTER AMPHETAMINE		
MOUSE	S	R	G	S	R	G	S	R	G
1	37	16	3	14	23	2	98	40	0
2	42	14	6	93	23	1	120	41	2
3	53	16	5	90	23	0	65	27	3
4	19	6	5	24	5	5	43	20	17
5	31	9	2	27	1	14	76	15	9
6	43	14	5	47	7	7	14	7	4
7	21	1	17	4	0	11	6	0	23
8	45	20	9	10	2	7	26	24	7
9	91	21	1	163	34	2	166	38	1
MEAN	42.4	13	5.87	52.4	13.1	5.44	68.2	23.5	7.33
				*	**		*	**	

* $p < 0.05$

** $p < 0.01$

S = Number of Squares
R = " " rearings
G = " " groomings

TABLE 9: OPEN FIELD BEHAVIOUR 30 MIN AFTER AMPHETAMINE (3.75MG) TREATMENT IN MICE PRETREATED WITH VEHICLE AND M. SALICIFOLIA 10 AND 25 MG/KG

	CONTROL			MS 10MG/KG			MS 25MG/KG		
MOUSE	S	R	G	S	R	G	S	R	G
1	14	23	2	5	0	0	7	0	1
2	93	23	1	2	0	2	51	1	0
3	90	23	0	174	0	0	5	0	1
4	24	5	5	65	0	0	121	4	0
5	27	1	14	20	1	5	14	0	0
6	47	7	7	51	8	3	3	0	2
7	4	0	11	8	1	14	8	0	3
8	10	2	7	52	4	2	41	3	0
9	163	34	2	6	0	3	21	1	0
MEAN	52.4	13.1	5.44	42.5	1.56	3.22	30.1	1.0	0.78
					*			*	**

* $p < 0.05$ (ANOVA)

** $p < 0.05$ (Multivalue or Nayman-Keul's test)

TABLE 10: OPEN FIELD BEHAVIOUR 60 MIN AFTER AMPHETAMINE 3.75MG/KG TREATMENT IN MICE PRETREATED WITH VEHICLE AND M. SALICIFOLIA 10 AND 25MG/KG

	CONTROL			MS 10MG/KG			MS 25MG/KG		
MOUSE	S	R	G	S	R	G	S	R	G
1	98	40	0	12	0	0	60	0	1
2	120	41	2	27	2	1	76	4	4
3	65	27	3	232	0	0	17	0	15
4	43	20	17	78	2	1	115	11	0
5	76	15	9	22	4	3	9	0	0
6	14	7	4	55	12	4	20	0	0
7	6	0	23	30	5	9	13	2	1
8	26	24	7	99	24	5	58	8	1
9	166	38	1	34	6	4	28	8	1
MEAN	68.2	23.5	7.33	65.4	6.11	3.0	44.0	4.89	2.44
					*			*	

* $p < 0.05$ (ANOVA)

MS = M. salicifolia extract
S = Number of squares
R = " " rearings
G = " " groomings

TABLE 11: OPEN FIELD BEHAVIOUR 60 MIN AFTER AMPHETAMINE (3.75MG/KG) ADMINSTRATION IN MICE PRE-TREATED WITH DIAZEPAM, HALOPERIDOL AND AMITRIPTYLINE

DRUG GIVEN 30 MIN BEFORE AMPHETAMINE	CONTROL			DIAZEPAM 10MG/KG			AMIPTRI-PTYLINE 10MG/KG			HALOPERIDOL 2MG/KG		
MOUSE	S	R	G	S	R	G	S	R	G	S	R	G
1	32	0	7	90	12	3	10*	0	2	18	0	3
2	70	11	2	134	0	0	5*	1	6	2	0	3
3	49	2	0	151	8	2	3*	0	4	9	0	4
4	44	3	3	51	0	0	14*	0	12	9	0	2
5	41	1	12	132	3	7	119	6	7	39	7	4
6	113	12	0	180	12	1	19	1	14	2	1	4
7	53	21	4	6	0	0	162	3	0	50	5	3
8	41	7	10	162	0	0	58	24	0	74	16	0
MEAN	52.7	7.1	4.7	113.2	4.4	1.6	48.8	4.4	5.6	25.4	3.6	2.9

* - Mice showing stereotyped sniffing.

S = number of squares

R = number of rearings

G = number of groomings

5.1.3.2 Amphetamine - induced lethality in aggregated mice

The results are shown in Table 12. One hour after treating mice with dexamphetamine (50mg/kg) intraperitoneally and keeping them in small cages in groups of ten, no deaths had occured in any of the groups. However, a qualitative change was observed. The mice in the control and the haloperidol (1mg/kg) pretreated group were very agitated and engaged in frequent noisy fighting. In contrast, mice pretreated with M. salicifolia 100mg/kg were much less agitated, fought only occasionally and made no noise.

By the 5th hour deaths had started occuring in all groups, and by the sixth hour 4 mice had died in both the control and the M. salicifolia pretreated groups but only two in the haloperidol pretreated group. At the end of 48 hours only 2 of the original 10 mice were still alive - 8 had died - in the control and M. salicifolia pretreated groups. Only 3 mice had died in the haloperidol pretreated group. Although all groups were kept at 10 mice by replacing dead mice with fresh ones only the mice which had been treated with amphetamine died.

In the M. salicifolia pretreated group death was preceded by hindlimb paralysis in most of the mice. Hind limb paralysis was not seen in the mice in the other groups.

TABLE 12: NUMBER OF DEAD MICE AFTER ADMINISTERING D-AMPHETAMINE (50MG/KG) IP AND KEEPING IN GROUPS OF 10

DRUG GIVEN BEFORE AMPHETAMINE	NUMBER OF DEAD MICE (OUT OF 10)					
	1hr	5hrs	6hrs	7hrs	24hrs	48hrs
CONTROL	0	3	4	4	7	8
MS 100MG/KG	0	3	4	6	7	8
HALOPERIDOL 1MG/KG	0	2	2	2	3	3

MS = M. salicifolia extract

TABLE 13: MEAN STEREOTYPY SCORES IN RATS AFTER INJECTION OF AMPHETAMINE 5MG/KG IP.

DRUG INJECTED 30 MIN BEFORE AMPHETAMINE	STEREOTYPY SCORE AFTER AMPHETAMINE INJECTION (MEAN) N=6									
	½hr	1hr	1½hr	2hr	2½hr	3hr	3½hr	4hr	4½hr	5hr
CONTROL	3	5	6	6	5	4	4	2	0	0
MS 100MG/KG	3	5	6	6	5	5	4	3	2	1
HALOPERIDOL 2MG/KG	0	0	0	0	0	0	0	0	0	0
AMITRIPTYLINE 10MG/KG	3	6	6	6	6	6	6	5	4	3

MS = M. salicifolia extract

Scoring scale for stereotypy

0 - Rat sleep or stationary

1 - Rat active

2 - Ambulatory with some sniffing

3 - Continuous sniffing with some ambulation

4 - Intensive continuous sniffing in one spot

5 - Continuous sniffing with some gnawing

6 - Continuous gnawing or licking

TABLE 14

MEAN STEREOTYPY SCORES IN RATS AFTER INJECTION OF APOMORPHINE (5MG/KG) SUBCUTANEOUSLY

DRUGS INJECTED 30 MIN BEFORE APOMORPHINE	STEREOTYPY SCORES AFTER APOMORPHINE INJECTION (MEAN) N=6					
	10MIN	20MIN	30MIN	40MIN	50MIN	60MIN
CONTROL	4	4	4	4	4	4
Ms (100MG/KG)	4	4	4	4	4	4
HALOPERIDOL (2MG/KG)	0	0	0	0	0	0

Scoring scale for apomorphine stereotypy

0 - normal behaviour

1 - exploratory activity, some sniffing

2 - continuous sniffing

3 - continuous sniffing, dicontinuous gnawing

4 - continuous gnawing.

MS = *M. salicifolia* extract

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5.1.4 TESTS FOR ANTIDEPRESSANT ACTIVITY

5.1.4.1 Amphetamine stereotypy

Results were shown on Table 13. In control animals amphetamine stereotypy lasted for 4 hours, the mean stereotypy scores had fallen to zero 4½ hours after amphetamine injection. Amitriptyline (10mg/kg) pretreated rats had mean stereotypy scores of 3 five hours after injection of amphetamine. This increase of the duration of stereotypy was expected of amitriptyline as an antidepressant (see discussion).

Five hours after amphetamine injection, the *M. salicifolia* extract treated rats had a mean stereotypy score of one, while thirty minutes before the mean score was two. In both cases the score for the control rats was zero. *M. salicifolia* extract (100mg/kg) seemed to cause a slight increase in duration of amphetamine stereotypy in rats indicating probable antidepressant activity.

5.1.4.2 Reserpine induced ptosis

Results are shown on Table 15.

In control mice the median palpebral ptosis score

two hours after reserpine (2.5mg/kg) injection was $^{2}/4$ ie eyes half-closed. The same was true of the mice treated with M. salicifolia extract (100mg/kg) 1 hour before reserpine. In mice pretreated with amitriptyline (10mg/kg) the median score was $^{0}/4$, indicating no significance ptosis.

M. salicifolia therefore seemed ineffective in protecting mice from reserpine induced ptosis.

TABLE 15: MEAN PTOSIS SCORES 2 HOURS AFTER INJECTING RESERPINE (2.5MG/KG) IN MICE

		DRUG		
	MOUSE	CONTROL	MS 100MG/KG	AMITRIPTLINE 10MG/KG
PTOSIS SCORES	1	2/4	4/4	0/4
	2	3/4	1/4	1/4
	3	1/4	1/4	0/4
	4	2/4	3/4	0/4
	5	2/4	2/4	0/4
	6	3/4	4/4	1/4
	MEDIAN	2/4	2/4	0/4

SCORING SCALE

0/4 - eyes fully open

1/4 - eyes slightly closed

2/4 - eyes half-way closed

3/4 - eyes almost fully shut

4/4 - eyes fully closed

MS = M. salicifolia extract

5.1.4.3 Behavioural despair in mice

The number of mobility scores, that is the number of times (out of ten) when the mouse was observed actively trying to swim out of the beaker were recorded for each of the 15 mice in each drug treatment group. The totals were then compared by normalized standardized deviate assuming Poison distribution.

Only in the group treated with amitriptyline 20mg/kg was the score greater than the control at the 5% level of significance. M. salicifolia extract (50 and 100mg/kg) and amitriptyline (100mg/kg) did not show antidepressant activity on this test. (Table 16).

TABLE 16: BEHAVIOURAL DESPAIR IN MICE - Number of Times, out of ten when each mouse was scored as mobile i.e. trying to swim out of beaker

MOUSE	CONTROL	AMI 10MG/KG	AMI 20MG/KG	MS 50MG/KG	MS 100MG/KG
1	1	1	2	2	4
2	1	5	3	2	2
3	1	4	6	2	4
4	4	1	8	3	6
5	1	9	5	3	7
6	6	6	1	2	1
7	5	4	5	4	2
8	1	0	3	2	5
9	3	3	10	1	5
10	4	6	6	0	7
11	10	2	4	2	5
12	6	4	2	5	2
13	1	1	6	3	4
14	3	9	7	5	3
15	4	5	8	5	3
TOTAL	49	60	76	42	60
MEAN	3.27	4.0	5.07	2.8	4.0
z value	1.0	1.05	2.41	0.73	1.05
p value		>0.2	<0.05	>0.2	>0.2

5.1.5 ANALGESIC ACTIVITY

5.1.5.1 Hot Plate Test

Results are shown on Table 17.

Compared with the reaction thresholds measured 18 hours previously. morphine hydrochloride increased the reaction time of mice by 4.23 ± 1.58 seconds and *M. salicifolia* extract (100mg/kg) by 7.32 ± 1.89 seconds. In the case of control mice, the reaction time fell by 1.04 ± 0.77 seconds. Using the F-ratio test, both morphine hydrochloride and *M. salicifolia* extract caused significant increases of the reaction time in mice at 5% level.

TABLE 17: REACTION TIMES OF MICE PLACED ON A HOT PLATE AT 58^{0}C, 18 HOURS BEFORE AND 30 MIN AFTER DRUGS

N = 10

DRUG	REACTION TIME 18 HRS PRE-DRUG	MEAN ± SEM	REACTION TIME 30 MIN AFTER DRUG	MEAN ±SEM	DIFFERENCE	MEAN ± SEM
CONTROL	6.5 7.1 6.1 5.5 6.7 9.8 6.6 7.1 9.2 6.5	7.11 ±0.43	4.5 7.8 2.9 8.7 6.6 7.8 4.7 8.9 4.2 4.6	6.07 ±0.67	-2.0 0.7 -3.2 3.2 -0.1 -2.0 -1.9 1.8 -5.0 -1.9	-1.04 ±0.77
MORPHINE 5MG/KG	2.9 3.2 9.9 6.0 3.9 4.8 3.7 9.5 5.2 6.1	5.52 ±0.78	9.2 8.5 8.1 8.4 7.7 7.4 20.2 10.3 10.8 6.9	9.75 ±1.22	6.3 5.3 -1.8 2.4 3.8 2.6 16.5 0.8 5.6 0.8	4.23 ±1.58 *
MS 100MG/KG	3.2 3.6 6.7 3.7 2.6 4.0 4.2 9.1 6.4 3.5	4.7 ±0.64	18.0 16.4 12.1 15.7 8.2 8.9 19.0 5.9 9.6 6.4	12.02 ±1.55	14.8 12.8 5.4 12.0 5.6 4.9 14.8 -3.2 3.2 2.9	7.32 ±1.89 **

* $p < 0.025$ (Using F ratio test)

** $p < 0.001$ (Using F ratio test)

MS = *M. salicifolia* extract

5.1.6 EFFECTS ON CHEMOCONVULSIONS

5.1.6.1 3-Mercaptopropionic Acid Convulsions

Results are shown in Table 19.

M. salicifolia extract at 100 and 200mg/kg had no effect against 3-MPA induced convulsions. Diazepam at 5mg/kg was only mildly effective, while at 10mg/kg diazepam protected most (5 out of 6) mice from 3-MPA convulsions. 3-MPA at the dose used produced tonic convulsions in all control mice. (Table 19)

5.1.6.2 Bemegride induced convulsions

Results are shown on Table 20.

M. salicifolia extract at 100mg/kg was ineffective against bemegride induced convulsions. Diazepam at 10mg/kg protected all mice tested (6) from bemegride convulsions. Bemegride at the dose used produced clonic convulsions in all the control animals.

5.1.6.3 Caffeine -induced convulsions

Results are shown in Table 21.

In control mice, caffeine (200mg/kg) intraperitoneally produced clonic and tonic convulsions accompanied by loss of postural control. Death followed within 30 minutes.

M. salicifolia extract at doses of 100 and 200 mg/kg intraperitoneally, did not affect the course of caffeine - induced convulsions and in all the mice treated with the extract, clonic and tonic convulsions preceded death which occured within 30 minutes.

In the mice treated with diazepam (10 and 20mg/kg), jumping and clonic convulsions occured in most of the mice but tonic convulsions and death did not occur.

TABLE 19: NUMBER OF MICE PROTECTED FROM 3 - MERCAPTOPROPIONIC ACID CONVULSIONS

DRUG	DOSE	n	PROTECTED FROM CLONUS	PROTECTED FROM TONUS
CONTROL	-	5	0	0
MS	100mg/kg	5	0	0
MS	200mg/kg	7	0	0
DIAZEPAM	5mg/kg	6	0	2
DIAZEPAM	10mg/kg	6	0	5

TABLE 20: NUMBER OF MICE PROTECTED FROM BEMEGRIDE CONVULSIONS

DRUG	DOSE	n	PROTECTED FROM CLONUS
CONTROL	-	6	0
MS	100mg/kg	6	0
DIAZEPAM	10mg/kg	6	6

TABLE 21: NUMBER OF MICE PROTECTED FROM CAFFEINE CONVULSIONS

DRUG	DOSE	n	PROTECTED FROM CLONUS	PROTECTED FROM TONUS	PROTECTED FROM DEATH
CONTROL	-	6	0	0	0
MS	100mg/kg	6	0	0	0
MS	200mg/kg	6	0	0	0
DIAZEPAM	10mg/kg	6	0	6	6
DIAZEPAM	20mg/kg	6	1	6	6

5.1.7 EFFECT ON PHYSOSTIGMINE LETHALITY

M. salicifolia 200mg/kg had no effect on the lethality of physostigmine sulphate (1mg/kg) in mice. All the mice whether pretreated with the vehicle or M. salicifolia extract died within 30 minutes of injection of physostigmine. Death in both groups was preceded by tremors, exophthalmos and salivation.

5.1.8 EFFECTS ON MOTOR PERFORMANCE

5.1.8.1 Chimney test

Results are shown on Table 22.

Only 4 out of 20 mice in the control group failed to climb the chimney (20cm up the inverted tube) within 30 seconds as opposed to 10 in the group injected with M. salicifolia extract(50mg/kg) and 17 in the group treated with 100mg/kg of the extract. The extract therefore showed a dose dependant impairement of the motor activity of mice.

5.1.8.2 The Traction Test

Results are shown in Table 23.

None of the mice injected with gum acacia 2% (the control group) failed the traction test, i.e. they all

grasped the wire with the hind feet within 10 seconds. In both the group treated with M. salicifolia extract 50 and 100mg/kg respectively, 9 out of 10 mice failed the traction test. This showed that the extract impaired the motor performance of mice though dose relationship was not shown in this case.

TABLE 22: NUMBER OF MICE FAILING CHIMNEY TEST i.e. COULD NOT CLIMB CHIMNEY WITHIN 30 SECONDS

(N=20)

DRUG ADMINISTERED BEFORE CHIMNEY TEST	MICE FAILING CHIMNEY TEST
CONTROL	4/20
MS 50MG/KG	10/20
MS 100MG/KG	17/20

MS = M. salicifolia extract

TABLE 23: NUMBER OF MICE FAILING TRACTION TEST

(N=10)

DRUG ADMINISTERED BEFORE TRACTION TEST	NUMBER OF MICE FAILING TRACTION TEST
CONTROL	0/10
MS 50MG/KG	9/10
MS 100MG/KG	9/10

5.1.9 INTERACTION WITH CNS DEPRESSANTS

5.1.9.1 Pentobarbitone sleeping time

Results are shown in Table 24.

M. salicifolia extract at 100mg/kg increases pentobarbitone sleeping time in mice by 60%. Mice pretreated with *M. salicifolia* extract slept for 100 ± 14.5 minutes while controls slept for 65 ± 3.8 minutes. This increase was significant to 5% level of confidence.

5.1.9.2 Ethanol sleeping time

Results are shown in Table 25.

M. salicifolia extract (100mg/kg) pretreated mice slept for 34 ± 6.26 minutes after 4g/kg ethanol while controls slept for 24 ± 4.40 minutes which was an increase of 42%. This was significant to the 5% level of confidence.

5.1.9.3 Ether anaesthesia

In paired rats, the member of the pair that was pretreated with *M. salicifolia* extract (100mg/kg) invariably had a longer duration of anaesthesia from the same exposure of diethyl ether than the control (Table 26). Anaesthesia was prolonged by 5.2 ± 0.65 minutes which was significant at 0.1% level of confidence.

TABLE 24: PENTOBARBITONE SLEEPING TIME - CONTROLS AND MICE PRETREATED WITH M. SALICIFOLIA (100MG/KG) N = 10

DRUG PRETREATMENT	SLEEPING TIME (MIN) AFTER PENTOBARBITONE (50MG/KG)	MEDIAN ± SEM. (MIN)
MS	168, >200, 74, 64, 100 110, 146, 61, 144, 90	100 ± 14.5 *
CONTROL	49, 59, 60, 71, 86, 63 69, 66, 61, 62	62 ± 3.82

* $p < 0.05$

TABLE 25: ETHANOL SLEEPING TIME, CONTROLS AND MICE PRETREATED WITH M. SALICIFOLIA (100MG/KG)

N = 10

DRUG PRETREATMENT	SLEEPING TIME (MIN) AFTER ETHANOL (4G/KG)	MEDIAN ± SEM (MIN)
MS (100MG/KG)	31, 36, 14, >200, 34 29 >200, 49, 28, 69	34 ± 6.26 *
CONTROL	27, 45, 8, 26, 35, 24, 17, 13, 25, 14	24 ± 4.40

* $p < 0.05$

TABLE 26 : DURATION OF ETHER ANAESTHESIA CONTROLS AND RAT PRETREATED WITH M. SALICIFOLIA EXTRACT (100MG/KG)

	RAT	MS 100MG/KG	CONTROL	DIFFERENCE
DURATION OF ANAESTHESIA (MIN)	1	13	7	6
	2	13	6	7
	3	10	6	4
	4	12	8	4
	5	10	6	4
	6	11	4	7
	7	8	4	4
	8	9	4	5
	9	8	6	2
	10	14	5	9
	MEAN ± SEM	10.8	5.6	5.2 * ± 0.65

* $p < 0.001$

5.1.10 EFFECT OF M. SALICIFOLIA EXTRACT ON BODY TEMPERATURE

5.1.10.1 Normal body temperature

Results are shown on Table 27.

When rats were injected with gum acacia suspension in sterile saline a slight fall in rectal temperature ($0.38 \pm 0.06^{o}C$) were observed two hours later, while there was a slight rise in temperature ($0.19 \pm 0.04^{0}C$) four hours later. These temperature changes were slight and reflected the temperature changes associated with the circardian rhythm. (Table 27)

A similar pattern of temperature changes was observed in the acetylsalicylic acid (100mg/kg) treated rats. Acetylsalicylic acid injection produced no change in body temperature relative to the controls.

When M. salicifolia extract (100mg/kg) was injected in rats intraperitoneally there was a sharp fall in rectal temperature: by $1.84 \pm 0.10^{0}C$ two hours after injection and $1.89 \pm 0.08^{0}C$ four hours after injection. This hypothermic effect is highly significant ($p < 0.001$).

In fact in some of the animals (3 out of 8) the rectal temperature had fallen below 35^0C, the lower limit of the clinical thermometer.

The test drugs had been injected at 10.00 hours and the rectal temperatures measured at 12.00 and 14.00 hours respectively. In this time the room temperature had risen from 20^0C to 25^0C and this rise may account for the high rectal temperatures in the control group at the 4 hour interval.

TABLE 27: EFFECT OF M. SALICIFOLIA EXTRACT AND ACETYLSALICYLIC ACID ON NORMAL RECTAL TEMPERATURE IN RATS

DRUG	RECTAL TEMP. BEFORE DRUGS (^0C)	RECTAL TEMP. 2 HRS AFTER DRUGS (^0C)	RECTAL TEMP. 4 HRS AFTER DRUGS (^0C)	TEMP. CHANGE AFTER 2 HRS (^0C)	TEMP. CHANGE AFTER 4 HRS (^0C)
CONTROL	37.3 37.4	36.7 37.2	37.7 37.5	-0.6 -0.2	+0.4 +0.1
	37.2 37.4	36.8 37.1	37.3 37.6	-0.4 -0.3	+0-1 +0.2
	37.9 37.2	37.3 37.0	38.0 37.4	-0.6 -0.2	+0.1 +0.1
	37.8 37.3	37.3 37.1	38.0 37.4	-0.5 -0.2	+0.2 +0.2
MEAN ± SEM	37.4 ± 0.01	37.1 ± 0.01	37.6 ± 0.01	-0.38 ± 0.06	+0.19 ± 0.04
ASA 100MG/KG	37.2 37.3	36.8 37.1	37.3 37.3	-0.4 -0.2	+0.1 0
	37.1 37.2	36.9 36.8	37.3 37.3	-0.2 -0.4	+0.2 +0.1
	37.0 37.2	36.7 36.9	37.1 37.4	-0.3 -0.3	+0.1 +0.2
	37.4 37.5	37.1 37.1	37.6 37.6	-0.3 -0.4	+0.2 +0.2
MEAN ± SEM	37.2 ± 0.003	36.9 ± 0.003	37.4 ± 0.003	-0.31 ± 0.06	+0.14 ± 0.03
MS 100MG/KG	36.8 37.0	35.4 35.1	35.0 35.2	-1.4 -1.9	-1.8 -1.8
	37.0 37.1	35.7 35.2	35.6 <35	-1.3 -1.9	-1.4 ->2.1
	37.1 37.0	<35 <35	<35 <35	->2.1 >2.0	->2.1 ->2.0
	37.1 37.1	<35 35.2	35.2 35.2	->2.1 -1.9	-1.9 -1.9
MEAN ± SEM	37.0 ± 0.001	35.2 ± 0.01	35.2 ± 0.01	-1.84 ± 0.10 *	-1.89 ± 0.08 *

* $p < 0.001$ ASA - Acetylsalicylic acid

MS - M. salicifolia extract

5.1.10.2 Antipyretic effect

Results are shown on Table 28.

Subcutaneous injection of brewer's yeast suspension 20 hours before had elevated the rectal temperature of the control group to 37.6 ± 0.05 ^{0}C from the normal 37^{0}C. Two hours and four hours later the rectal temperature of the rats had risen to 38.4 ± 0.03 ^{0}C, indicating that the pyretic effect of the yeast was still getting more pronounced.

By comparison the rectal temperature of the acetylsalicylic acid (100mg/kg) treated rats had fallen by 0.31 ± 0.14 ^{0}C two hours and 0.61 ± 0.09 ^{0}C four hours after acetylsalicylic acid injection respectively. Overall, the temperature had fallen from 37.8 ± 0.01 ^{0}C before injection to 37.2 ± 0.03 ^{0}C four hours after injection. Compared with the control group this temperature fall was highly significant ($p < 0.001$) and attests to the antipyretic effects of acetylsalicylic acid.

The temperature fall in pyretic rats was even more dramatic after M. salicifolia extract injection falling by 2.03 ± 0.15 two hours and 2.16 ± 0.13 ^{0}C four hours after injecting the extract.

This antipyretic effect was highly significant ($p < 0.001$). The mean rectal temperature had fallen from $38.1 \pm 0.03\ ^0C$ before injection to $36.1 \pm 0.41\ ^0C$, which was well below the normal 37^0C, four hours after injection.

TABLE 28 EFFECT OF M, SALICIFOLIA EXTRACT AND ACETYLSALICYLIC ACID ON THE RECTAL TEMPERATURES OF PYRETIC RATS

DRUG	RECTAL TEMP. BEFORE DRUGS (^0C)	RECTAL TEMP. 2HRS AFTER DRUGS (^0C)	RECTAL TEMP. 4HRS AFTER DRUGS (^0C)	TEMP. CHANGE AFTER 2 HRS (^0C)	TEMP. CHANGE AFTER 4 HRS (^0C)
CONTROL	38.0 37.65	38.3 38.5	38.3 38.7	+0.3 +0.85	+0.3 +1.05
	37.5 37.35	37.4 38.1	37.5 38.0	-0.1 +0.75	0 +0.65
	37.75 37.75	38.9 38.9	38.9 38.9	+1.15 +0.90	+1.15 +0.90
	37.5 37.65	38.5 38.5	38.5 38.7	+1.0 +0.65	+1.0 +0.70
MEAN ± SEM	37.6 ± 0.05	38.4 ± 0.03	38.4 ± 0.03	0.69 ± 0.144	0.72 ± 0.14
ASA	37.6 38.3	37.0 37.9	37.0 37.3	-0.6 -0.4	-0.6 -1.0
	37.7 37.6	38.0 37.9	37.6 37.1	+0.3 +0.3	-0.1 -0.5
100MG/KG	38.15 37.9	37.7 37.2	37.6 37.2	-0.45 -0.7	-0.55 -0.7
	37.45 37.6	36.9 37.0	36.6 37.0	-0.55 -0.4	-0.85 -0.6
MEAN ± SEM	37.8 ± 0.01	37.5 ± 0.01	37.2 ± 0.03	-0.31 ± 0.14*	-0.61 ± 0.09*
MS	37.9 37.95	36.4 35.9	35.5 36.1	-1.5 -2.05	-2.4 -1.85
	38.1 38.95	36.4 36.0	36.0 36.1	-1.7 -2.95	-2.1 -2.85
100MG/KG	38.15 37.8	36.3 35.9	35.9 36.7	-1.85 -1.9	-2.25 -2.1
	38.45 37.75	36.2 35.7	36.3 36.2	-2.25 -2.05	-2.15 -1.55
MEAN ± SEM	38.1 ± 0.03	36.1 ± 0.09	36.1 ± 0.41	-2.03 ± 0.15*	-2.16 ± 0.13*

* $p < 0.001$

ASA - Acetylsalicylic Acid

MS - M. salicifolia extract

5.2 EFFECTS OF M. SALICIFOLIA EXTRACT ON THE GASTRO-INTESTINAL TRACT

5.2.1 Guinea-pig ileum

Results are shown in Figs 9a, b, c, d.

M. salicifolia extract caused contraction of the guinea-pig ileum. These contractions were blocked by the addition of atropine sulfate (600 µg) to the organ bath 2 minutes before the M. salicifolia extract. (Fig. 9b)

Hexamethoniun bromide and mepyramine maleate had no effect on M. salicifolia - induced contractions (Figs 9c, d).

5.2.2 Rabbit jejunum

Results are shown in Fig 10a, 10b)

M. salicifolia extract increases the tone and amplitude of the intrinsic contractions of the rabbit jejunum. (Fig 10a, 10b)

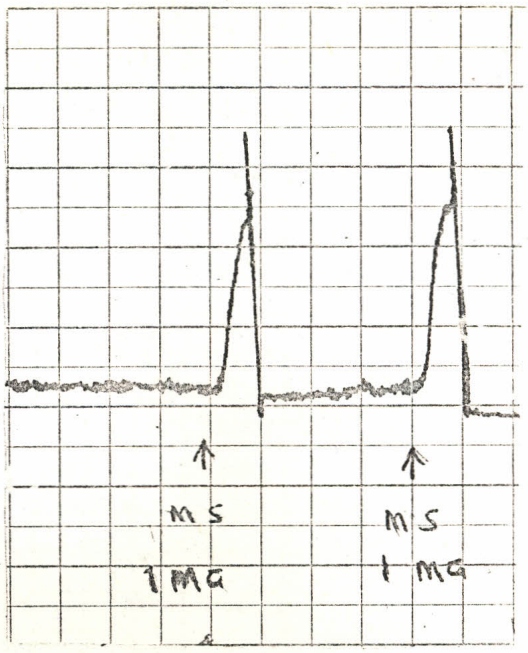


Fig 9(a) Effect of M. salicifolia extract (1mg) on Guinea pig ileum.

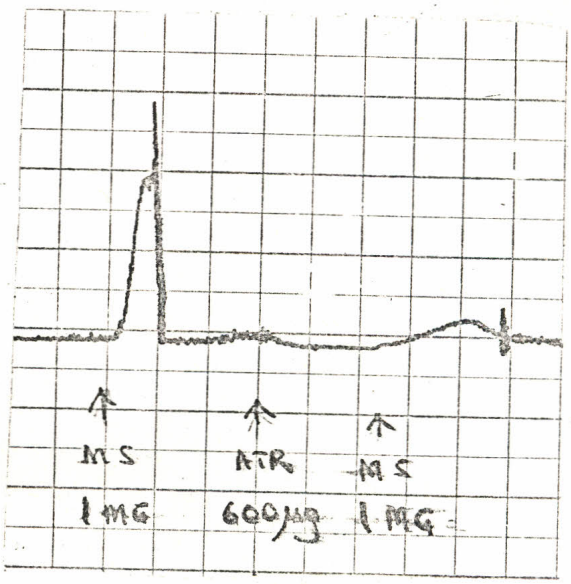


Fig 9(b) Effect of atropine sulfate (600µg) on M. salicifolia extract (1mg) contractions in guinea pig ileum when added to organ bath 2 minutes before

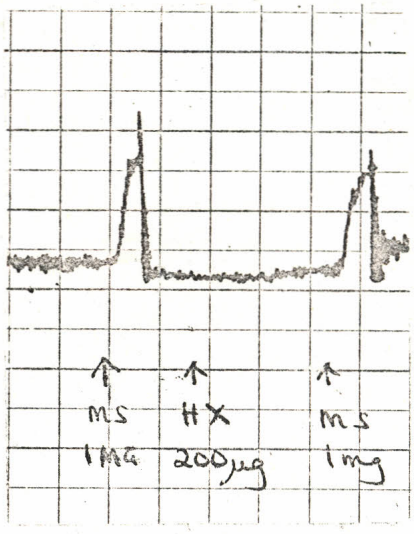


Fig 9(c) Effect of hexamethonium bromide (200µg) on M. salicifolia (1mg) contractions in guinea pig ileum when added to organ bath 2 minutes before

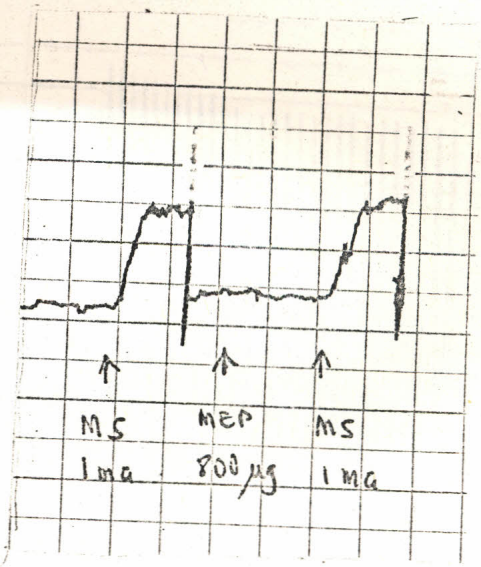


Fig 9(d) Effect of mepyramine maleate (800µg) on M. salicifolia (1mg) contractions in guinea pig ileum when added to organ bath 2 minutes before.

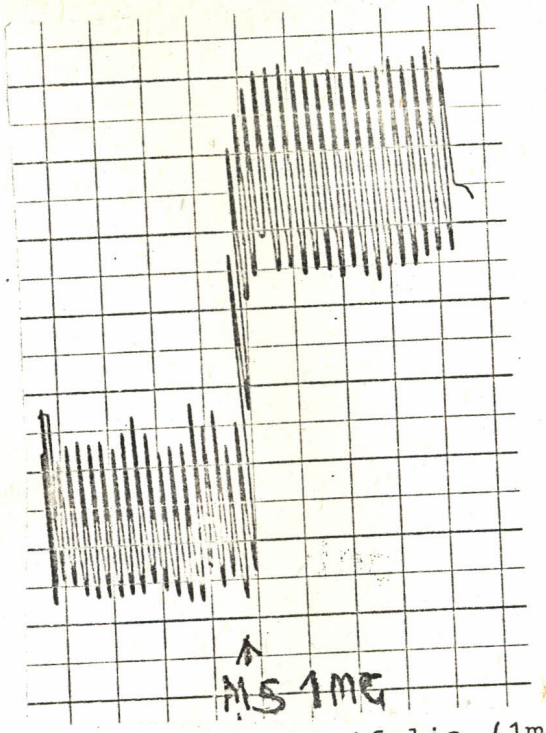


Fig 10a Effect of M. salicifolia (1mg) on the contractions of the rabbit jejunum

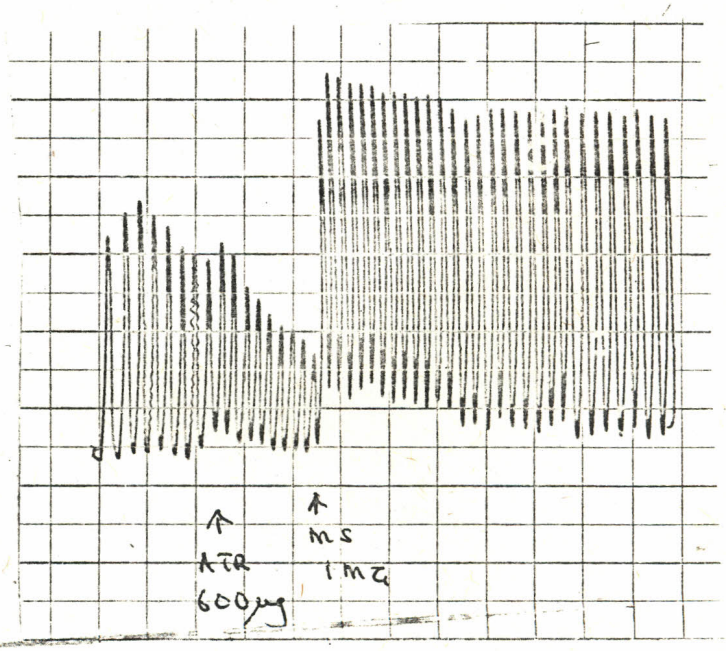


Fig 10b Effect of atropine sulfate (600µg) on the M. salicifolia (1mg) contractions in the rabbit jejunum.

5.2.3. Gut Motility in vivo

a) Effects in the rat

(i) When administered orally *M. salicifolia* extract at doses of 30 and 100mg/kg caused a slight reduction in the distance moved by the charcoal meal through the rats' intestines (Table 29). This reduction was not statistically significant.

(ii) When administered intraperitoneally *M. salicifolia* extract 100mg/kg caused a 77.7% reduction in the distance moved by the charcoal meal through the rats' intestine as compared with the controls (Table 30).

This reduction was significant at the 0.1% level of confidence. It was also noted when dissecting the animals to reach the intestines that there was a lot of fluid in the peritoneal cavity in the *M. salicifolia* treated animals.

b) Effect in mice

Results are shown in Table 31.

When administered intraperitoneally in mice *M. salicifolia* extract 200mg/kg caused a 79% reduction in the distance moved by the charcoal meal through the mice's intestines.

In comparable conditions codeine sulfate 40mg/kg caused a 21% reduction in distance moved by the charcoal meal. There was a lot of fluid in the peritoneal cavity of the M. salicifolia treated animals.

TABLE 29: EFFECT OF M. SALICIFOLIA ON GIT MOTILITY IN THE RAT WHEN ADMINSTERED ORALLY

(Ratio of Distance moved by charcoal (CD) to the length of the intestines (IL).

N = 7

	CD/IL RATIOS (x100)	MEAN ± SEM	P value
CONTROL	77.8, 59, 68, 68.5, 71.6, 73.6, 66.9	69.34 ± 2.24	
MS 30MG/KG (ORALLY)	49, 61, 70.5, 56 64.9, 72, 78	64.48 ± 3.78	>0.5
MS 100MG/KG (ORALLY)	63.3, 61.9, 48.6, 56, 69.3, 71	62.6 ± 3.03	>0.5

TABLE 30: EFFECT OF M. SALICIFOLIA ON GIT MOTILITY IN THE RAT WHEN INJECTED INTRAPERITONEALLY

Ratio of Distance moved by charcoal (CD) to length of intestines (IL)

N = 7

	CD/IL RATIOS (x100)	MEAN ± SEM	P value
MS 100MG/KG (IP)	1.5, 19.0, 17.4, 20.8, 10.5, 11.8, 15.8	13.83 ± 2.48	<0.001
CONTROL	72.1, 56.9, 59.5, 70.9, 41.2, 71.6, 62.1	62.04 ± 4.19	

TABLE 31: EFFECT OF M. SALICIFOLIA ON GIT MOTILITY IN THE MOUSE WHEN ADMINSTERED INTRAPERITONEALLY

Ratio of Distance moved by charcoal (CD) to length of the intestine (IL). N = 8

	CD/IL RATIOS (x100)	MEAN ± SEM	P value
CONTROL	90, 69, 93, 64, 75 80, 68, 100	80 ± 4.7	
MS 200MG/KG (IP)	0, 38, 26, 0, 22, 3, 33, 14	17 ± 5.3	<0.01
CODEINE 40MG/KG (IP)	58, 72, 59, 51, 29 72, 78, 83	63 ± 6.2	<0.01

5.2.4 Ulcerogenic effects

On visual examination of the stomachs of the rats treated orally with *M. salicifolia* extract (200mg/kg) and indomethacin (10mg/kg) after fasting for 24 hours showed evidence of corrosion of the stomach wall. This was further confirmed when the stomachs were examined under a dissecting microscope (Will Wetzlak, Germany) at a ten factor (x10) magnification. In this case long eroded valleys were observed in the stomach walls.

None of the control rats had any evidence of stomach corrosion.

5.3. EFFECTS OF M. SALICIFOLIA ON THE CARDIOVASCULAR SYSTEM

5.3.1 Rat Blood pressure

Results are shown in Figs 11 - 14.

Administration of 0.25ml saline to the anaesthetised rat through the femoral vein resulted in a slight transient rise in blood pressure (Fig 11). This was a volume effect.

Administration of M. salicifolia extract (2mg) in 0.25ml saline produced a sustained fall in blood pressure (Fig 11). Repeated administration of the extract at the same dosage produced a fall in heart rate as well (Fig 12). This was accompanied by a fall in respiration rate and the animal breathe very forcefully.

Administration of acetylcholine (0.25µg) caused a fall in blood pressure (fig 13). When the rat was atropinised with 240µg atropine sulfate (Fig 11) acetylcholine no longer produced a fall in blood pressure (Fig 14). The hypotensive effect of M. salicifolia extract (2mg) was not affected however (Fig 14).

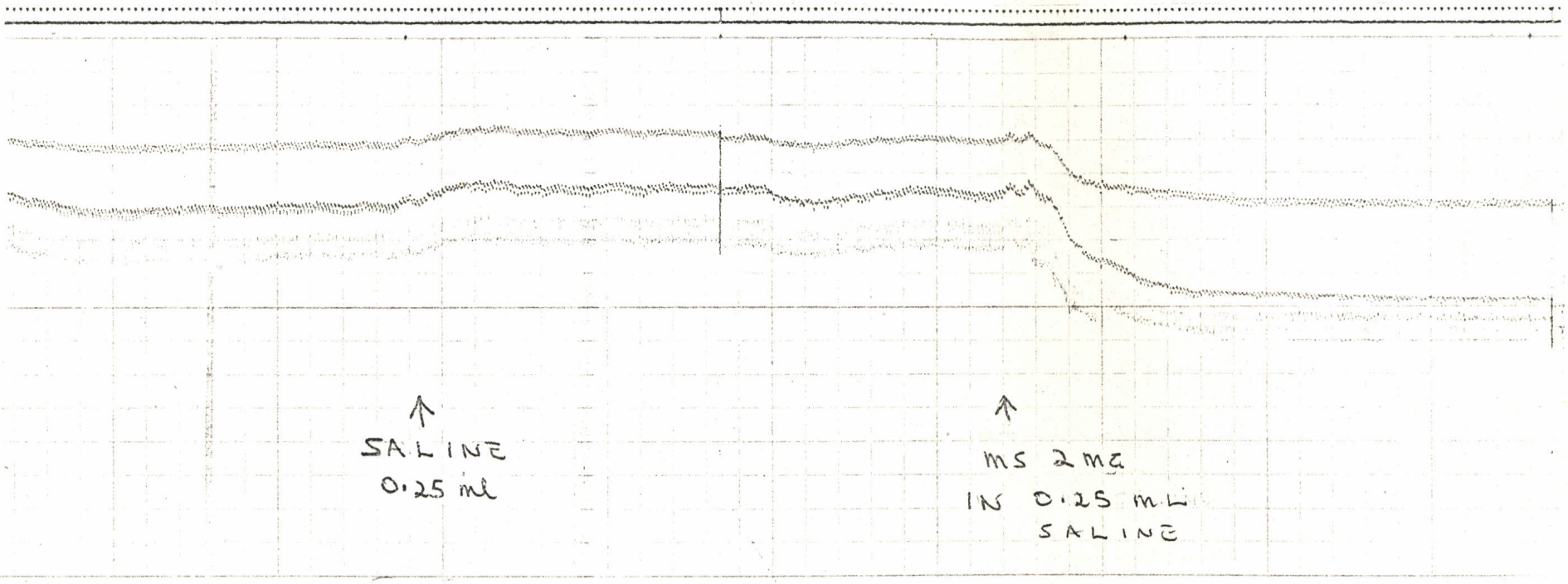


Fig 11: The effects of adminstering saline (0.25ml) and M. salicifolia extract (2.0mg) to blood pressure of the anaesthetised rat.

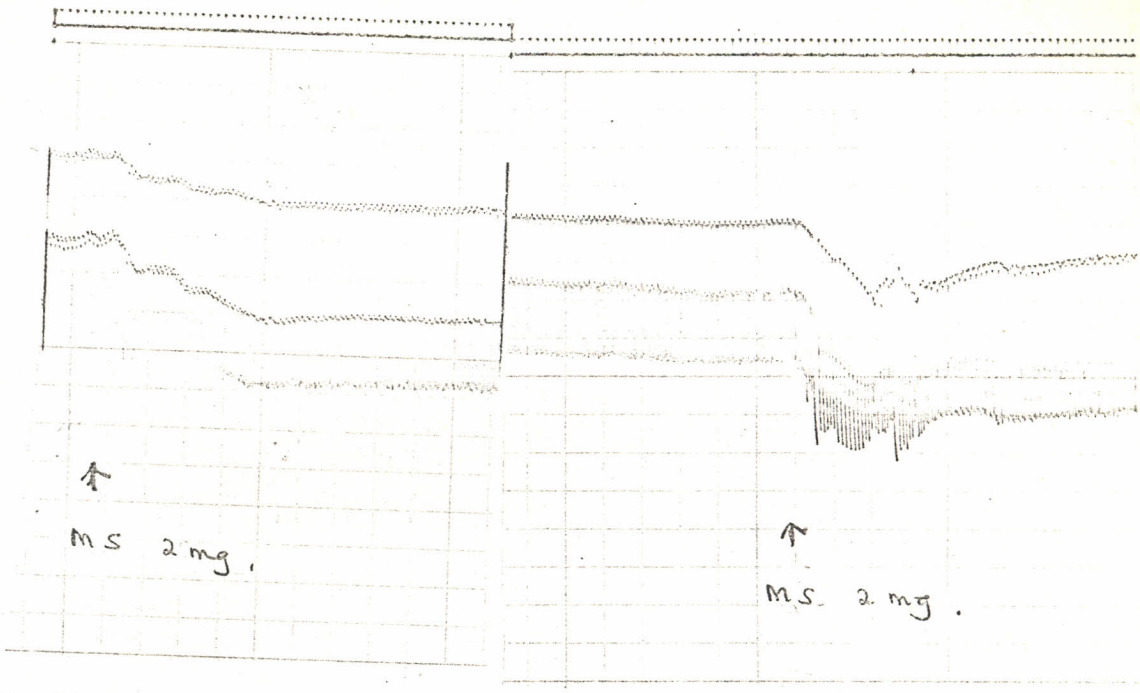


Fig 12: The effect of repeated administration of M. salicifolia extract (2mg) on the heart rate of the anaesthetised rat. Note the fall in heart rate as well as blood pressure.

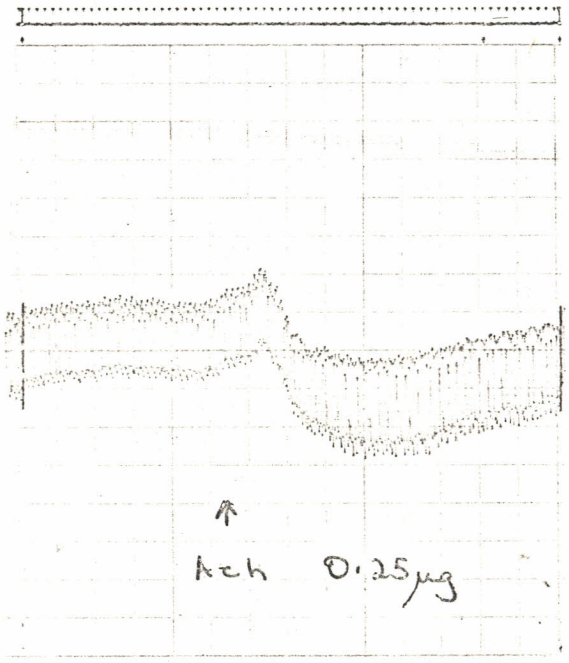


Fig 13: The effect of acetylcholine (0.25µg) on the blood pressure of the anaesthetised rat.

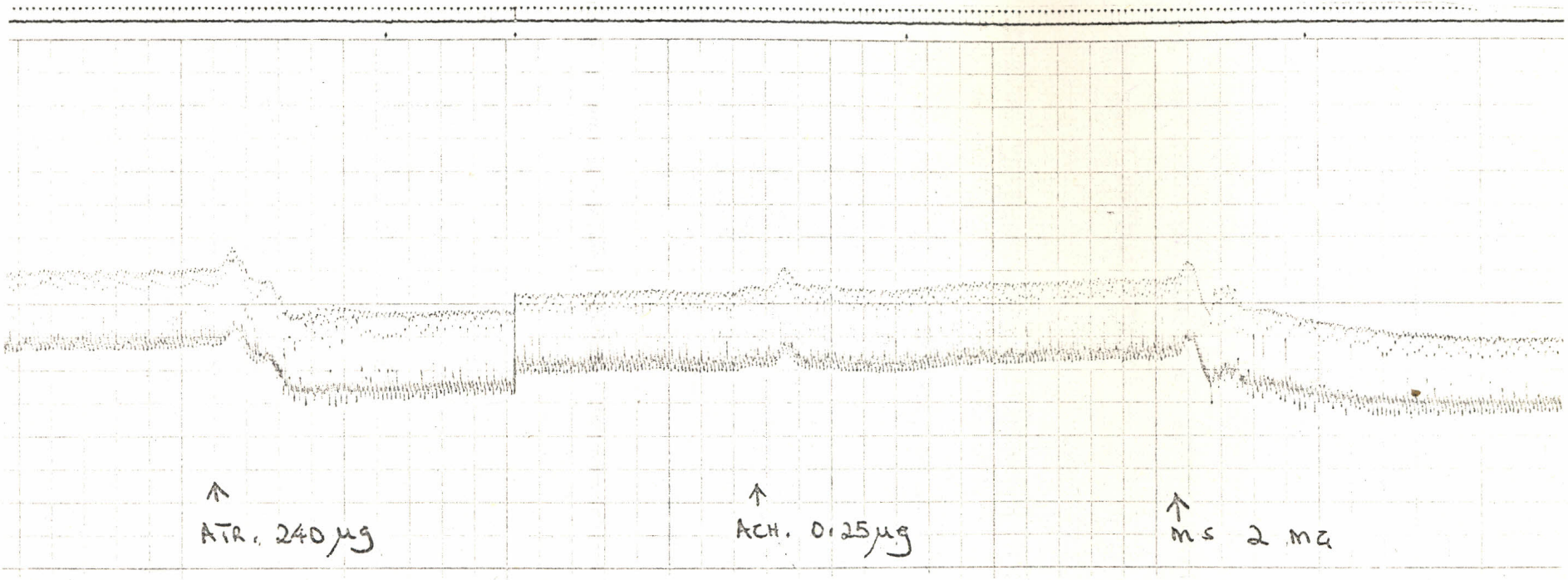


Fig 14: The effects of Atropine (240µg) and subsequently acetycholine (0.25µg) and M salicifolia extract (2mg) on the blood pressure of the anaesthetised rat.

5.3.2 Isolated perfused rabbit heart

Results are shown in Fig 15.

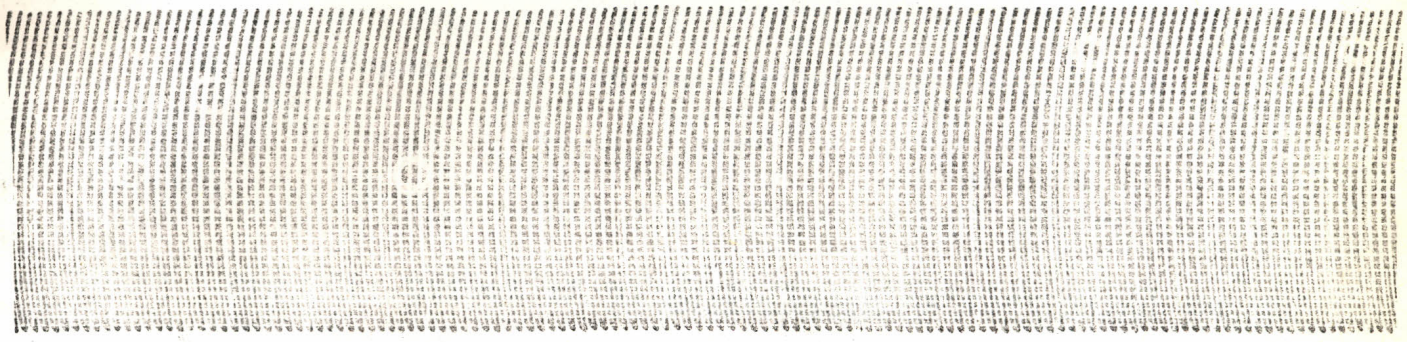
M. salicifolia extract (2-20mg) had no effect on the rate or force on contraction of the isolated perfused rabbit heart.

5.3.3 Perfused rat hindquarters

Results are shown in Fig 16 and 17.

M. salicifolia extract (4mg) produced a rise in perfusion pressure of the rat hindquarters. (Fig 16) Adrenaline (10µg) caused a rise in the perfusion pressure while acetylcholine (2µg) cause a fall. (Fig 17)

M. salicifolia extract unlike adrenaline had no perceptible effect on the perfusion volume. Adrenaline virtually stopped the flow of the perfusion fluid, and in fact rendered the preparation edematous, thereby reducing its usefulness.



↑
M. S
10 mg.

Fig 15: Effect of M. salicifolia extract (10mg) on the isolated perfused rabbit heart

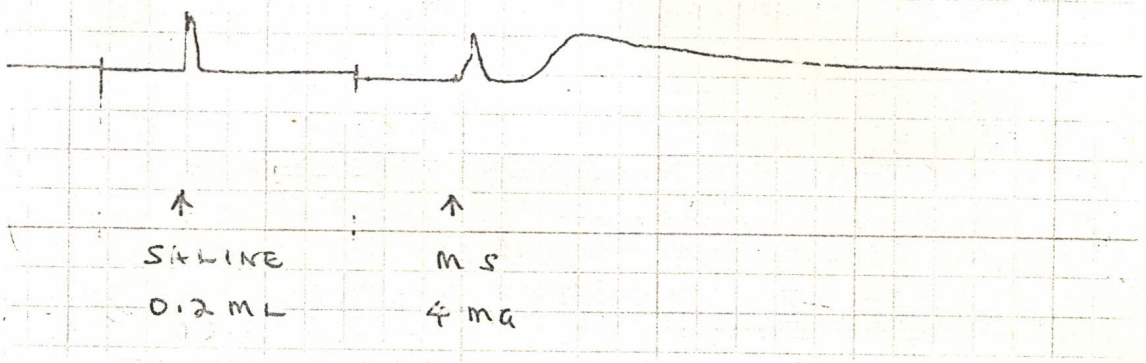


Fig 16: Effect of M. salicifolia extract (4mg) on the perfusion pressure of the perfused rat hindquarters.

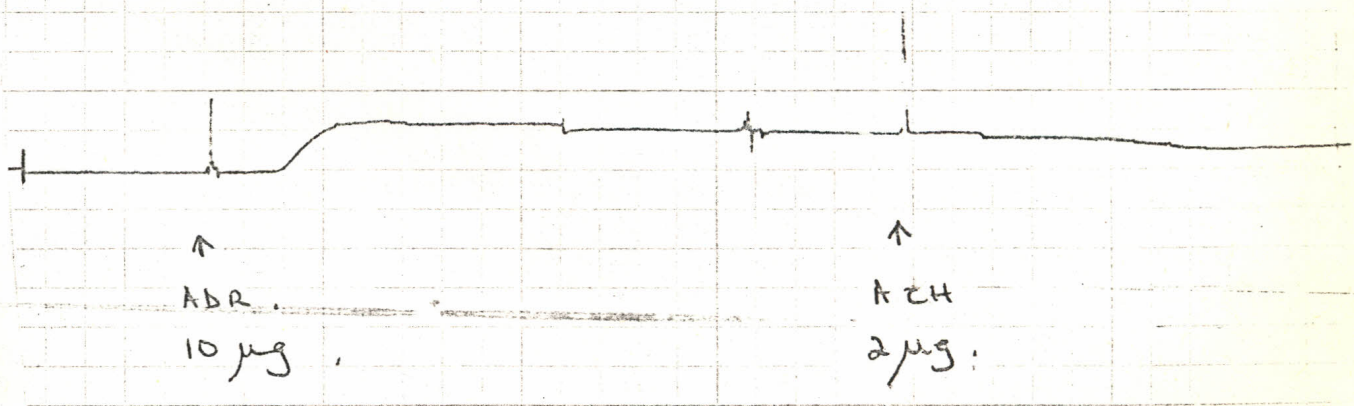


Fig 17: Effect of adrenaline (10µg) and acetylcholine (2µg) on the perfusion pressure of the rat hindquarters

5.4. EFFECT OF M. SALICIFOLIA EXTRACT ON OTHER ORGAN SYSTEMS

5.4.1 Diuresis in the rat

Results are shown in Table 32.

M. salicifolia extract injected intraperitoneally in rats (100mg/kg) completely inhibits urine production.

On killing those rats injected with M.salicifolia extract the peritoneal cavity was found to contain a lot of fluid, the bladder was empty while the blood was very viscous. The extract appeared to draw fluid from the blood so that it accumulated in the peritoneal cavity. No other differences from control rats were noticed.

Administered orally, M. salicifolia extract (100 and 200mg/kg) did not significantly change urine output relative to the controls. By comparison, frusemide the standard diuretic used increased urine output by 50% which was highly significant ($p < 0.001$).

TABLE 32 : DIURESIS IN THE RAT URINE OUTPUT OVER 6 HRS, 1 HR AFTER TEST DRUGS

DRUG (ROUTE)	N (pairs of rats	6 HR URINE VOL(ML)	MEAN ± SEM	p value
CONTROL (IP)	3	40.3 36.3 38.2	38.3 ± 1.16	
MS 100MG/KG (IP)	3	0 0 0	0	
FRUSEMIDE 20MG/KG (IP)	3	58.7, 63 60.8	60.8 ± 1.24	< 0.001
CONTROL (ORAL)	5	29, 39.5, 40, 31, 36	35.1 ± 2.22	
MS 100MG/KG (ORAL)	4	40.5, 47, 47, 45	44.9 ± 1.53	> 0.5
MS 200MG/KG (ORAL)	5	31, 43, 36 36, 42, 38	38 ± 2.17	> 0.5

MS - *M. salicifolia* extract

5.4.2 Antiinflammatory Effects - Carrageenan - Induced paw Edema

Results are shown in Table 33.

The mean swelling of the rat paws 4 hours after injection of Carrageenan was 0.476 ± 0.42 ml for the control rats, 0.433 ± 0.97ml for those rats pretreated with *M. salicifolia* extract (200mg/kg) and 0.055 ± 0.376 for those pretreated with indomethacin (10mg/kg).

Indomethacin significantly reduced the degree of carrageenan - induced paw edema ($p < 0.01$) but *M. salicifolia* extract was inactive ($p > 0.4$).

TABLE 33: CARRAGEENAN - INDUCED PAW EDEMA - INCREASE IN PAW VOLUME 3 HRS AFTER CARRAGEENAN INJECTION (n = 6)

DRUG	INCREASE IN PAW VOLUME (ML)	MEAN ± SE	P
MS 200MG/KG	0.69 0.59, 0.46 0.46, -0.05 0.40	0.433 ± 097	> 0.4
IND 10MG/KG	0.22, 0.11 0. , -0.01 -0.07 0	0.055 ± 0.386	< 0.01
CONTROL	0.42, 0.63 0.36, 0.41 0.47 0.57	0.476 ± 0.42	

5.4.3 Local anaesthesia - guinea pig wheal test

Results are shown on Table 34.

Pin-pricks on wheals in the guinea-pigs formed from injecting vehicle (gum acacia 2%) intradermally, invariably produced twitching. The same was true of wheals produced by *M. salicifolia* (10mg/ml) injection. On the other hand the number of twitches produced on pricking the wheals formed by injection of the standard local anaesthetic procaine hydrochloride (3mg/ml) was much reduced.

TABLE 34 : NUMBER OF PINPRICKS (OUT OF 6) THAT DID NOT RESULT IN TWITCHES IN WHEALS IN THE GUINEA PIG

DRUG	WHEAL NO.	0 MIN	5 MIN	10 MIN	15 MIN	20 MIN	25 MIN	30 MIN	DURATION OF ANAESTHESIA
SALINE	1	0	0	0	0	0	0	0	NIL
	2	0	0	0	0	0	0	0	
	3	0	0	1	0	0	0	0	
	4	0	0	0	0	0	0	0	
	5	0	0	0	0	0	1	0	
	MEAN	0	0	0.2	0	0	0.2	0	
MS 10MG/ ML	1	0	1	0	0	0	0	0	NIL
	2	0	0	0	0	0	0	0	
	3	0	0	0	0	0	0	0	
	4	1	0	0	0	0	0	0	
	5	0	0	0	0	0	1	0	
	MEAN	0.2	0.2	0	0	0	0.2	0	
PROCA-INE 3MG/ML	1	6	6	6	5	2	4	3	> 30 MIN
	2	6	6	6	4	2	2	1	> 30 MIN
	3	0	3	1	2	1	2	2	> 30 MIN
	4	6	2	4	2	1	0	0	20 MIN
	5	1	5	6	6	5	6	5	> 30 MIN
	MEAN	3.8	4.4	4.6	3.8	2.2	2.8	2.2	

MS - *M. salicifolia* extract

5.4.4 Rat phrenic nerve diaphragm

M. salicifolia extract (2 - 10mg) produced no effects on the amplitude of the contractions of the rat diaphragm produced by electrical stimulation of the nerve (Fig 18).

5.4.5 Guinea-pig trachea

Results are shown on Table 3.5.

M. salicifolia extract produce contractions of the guinea-pig trachea. The degree of contraction, as measured by rise of fluid in the capillary tube was reduced by adminstration of atropine (2.0 µg) 3 minutes before the introduction of *M. salicifolia* extract into the organ bath. Atropine caused slight relaxation of the trachea

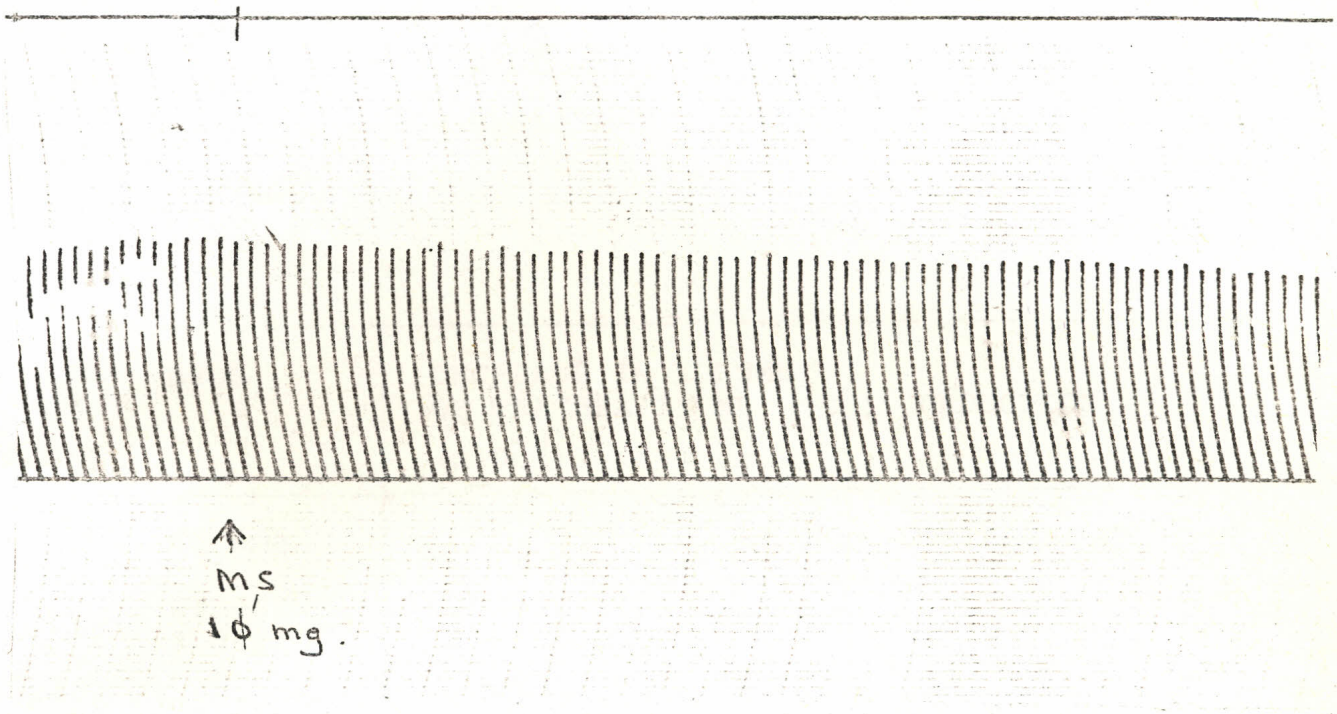


Fig 18: Effect of M. salicifolia extract on the neuromuscular junction (rat phrenic nerve - diaphragm preparation)

TABLE 35: EFFECT OF M. SALICIFOLIA EXTRACT ON THE INTACT GUINEA PIG TRACHEA BEFORE AND AFTER ATROPINE SULFATE (AS INDICATED BY VOLUME CHANGES)

DRUG	DECREASE IN VOLUME OF TRACHEA IN ML ($x10^{-3}$)	DEDUCTION
MS 2mg	2.5	CONTRACTION
ATROPINE 2.0µg	-0.2	RELAXATION
MS 2mg	2.5	CONTRACTION
ATROPINE 2.0µg + MS 2mg	1.5	CONTRACTION REDUCED
MS 1mg	1.5	CONTRACTION
ATROPINE 2µg + MS 2mg	1.0	CONTRACTION REDUCED
MS 2mg	2.0	CONTRACTION
ATROPINE 2µg + MS 2mg	1.0	CONTRACTION REDUCED
MS 2mg	1.5	CONTRACTION
ATROPINE 2µg + MS 2mg	0.7	CONTRACTION REDUCED

5.4.6 Rat uterus

Results are shown on Fig 19.

M. salicifolia extract (2mg) produced contraction of the rat uterus. However these contractions are blocked by introduction of atropine (1.2μg) into the organ bath 2 minutes before the introduction of M. salicifolia extract. This indicated that the contractions were cholinergic rather than oxytocic.

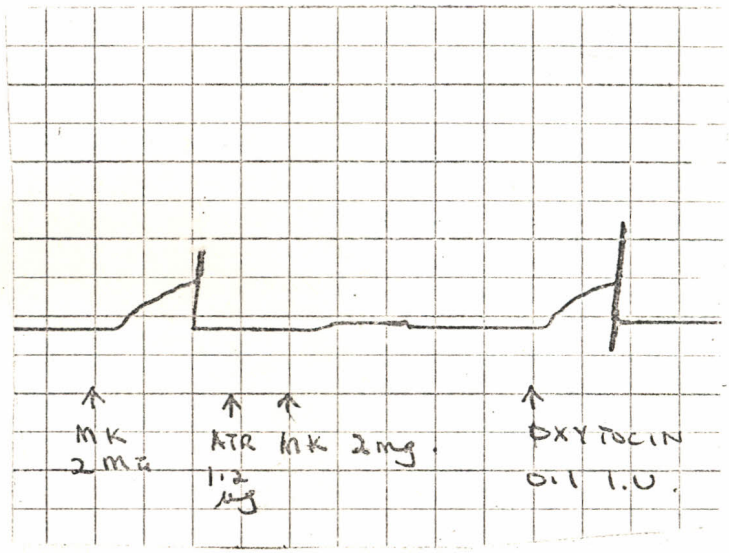


Fig 19: Effect of M. salicifolia extract (2mg) on the rat uterus, before and after addition of atropine (1.2µg) in the organ bath.

5.5 ACUTE TOXICITY

Results are summarised in Table 36.

The number of deaths 24 hours after injecting *M. salicifolia* extract ranged from $^{1}/10$ at 100 and 125.9 mg/kg to $^{10}/10$ at 501 mg/kg. The survivors were huddled together and looked ill and most of them had total palpebral ptosis. The median lethal dose (LD_{50}) was found to be 248 ± 22 mg/kg.

TABLE 36: ACUTE TOXICITY OF M. SALICIFOLIA EXTRACT - NUMBER OF MICE DYING WITHIN 24 HOURS AFTER VARIOUS DOSES OF THE EXTRACT

DOSE MG/KG	LOG DOSE	NO. OF DEAD MICE (OUT OF 10) IN 24 HRS	% DEAD 24 HRS	NO. OF DEAD MICE (48 HRS)
100	2.000	1	10	3
125.9	2.100	1	10	4
158.5	2.200	3	30	6
199.5	2.300	9	90	10
251.2	2.400	6	60	8
316.2	2.500	8	80	10
398.1	2.600	9	90	10
501.2	2.700	10	100	10

Chapter six

DISCUSSION

Part 1: Phytochemical Investigation of Myrica salicifolia root extract

Screening tests indicated that M. salicifolia root extract contains at least six phenolic compounds, a yellow pigment and traces of terpenoid compounds. Other than tannins no other chemical groups were detected. The absence of alkaloids is especially notable since most psychoactive plants - coca, opium, tobacco etc - owe their activity to alkaloids.

Since the tests for flavonoids and anthraquinones were negative, it is most likely that the phenolic compounds found in M. salicifolia roots are phenylpropanoids. This is suggested by the fact that these compounds are lipid - soluble, they were eluted from the silica gel column with petroleum ether and chloroform. Phenylpropanoids are naturally occuring phenolic compounds with an aromatic ring attached to a three - carbon side chain and include the coumarins, phenylpropenes and lignans. The phenylpropanoids are unique among the phenolic compounds in being lipid-soluble (Harborne 1973).

The most lipophilic of the M. salicifolia compounds, MS(A) was isolated. Judging from the intensity of the TLC spots it was the principal component. The infra-red spectrum obtained indicated that MS(A) has an aromatic nucleus and a cyclic carbonyl group. These two groups together with the phenolic group detected on TLC suggest that the compound is likely to be a phenylpropanoid, most probably a coumarin derivative. The ultraviolet (uv) spectrum obtained was difficult to interpret. Further work to obtain the nuclear magnetic resonance (NMR) and mass spectrometry data would be necessary before the molecular structure of the compound can be elucidated.

When isolated, MS(A) was soluble in petroleum ether but insoluble in ethanol, methanol and water. This is interesting since MS(A) was obtained from a methanolic extract, which was fairly soluble in ethanol and water. It indicates that the other components of the extract help in solubilising the compound.

It was not possible to establish if MS(A) had any pharmacological activity as the quantity obtained was too small and in any case it was not possible to solubilise it in a physiologically inert solvent for administration to animals or isolated tissues.

With its high lipid-solubility MS(A) would easily penetrate the blood brain barrier and express effects in the brain.

Further work would be necessary to determine what compound(s) in the extract contribute to its pharmacological activity.

A number of phenylpropanoids are known to have effects on the central nervous system. For instance, angelicin, a coumarin isolated from Selinum vaginatum possesses potent tranquillosedative, anticonvulsant and central muscle relaxant properties (Chandhoke and Ghatak 1975). The hallucinogenic effects of the nutmeg (Myristica fragans) have been attributed to the phenylpropene, myristicin (Schultes 1978).

It is worth noting that non-flavonoid phenolic compounds have been isolated from other plants of the genus Myrica. Malterud et al (1976) isolated two new metaperacyclophanes, galeon and hydroxygaleon from M. gale. There are no reports on the biological activity of these compounds. Terpenoids have also been reported. Matyukhina et al (1978) isolated myricardiol, taraxerol and taraxerone from the roots of M. tomentosa, while Paul et al (1974) isolated the same three compounds from M.cerifera roots, The biological activity of the compounds was not reported.

It is possible that these compounds were among those detected on thin layer chromatograms of M. salicifolia root extract.

The methanolic extract of M. salicifolia root was used for the pharmacological investigations. For this to be done it was necessary to define the extract in such a way as to ensure that it was the same entity that was being evaluated all the time. A pharmaceutical entity must be defined both qualitatively and quantitatively.

The thin layer chromatograms of a plant extract on several TLC systems are characteristic. Therefore, an extract can be identified as being from M. salicifolia root if on running TLC's in several solvent systems the same number of spots, Rf values and relative intensities were obtained as with an authenticated M. salicifolia extract in the same conditions.

The absorbance in ultraviolet light of a specified concentration of M. salicifolia methanolic extract was used as a criterion for quantitation. For instance, the absorbance of a 1% methanolic solution of M. salicifolia extract in a 1cm cell ($E^{1\%}_{1cm}$) at 211nm is 791. (See chapter four)

A methanolic extract of M. salicifolia roots which gave this absorbance (and the same TLC profile) would be expected to give the same pharmacological activity reported in this work.

Once qualitatively and quantitatively defined, M. salicifolia extract could be used for pharmacological evaluation. That it is a combination of many compounds is no longer material. It is a fact that a large number of natural drug products that are in essence complex matrices - senna tablets, insulin injections, ipecac, Rauwolfia serpentina tablets and deglycyrrhizinised liquorice - stand beside purified synthetics in the armamentrium of modern medicine.

Part 2: Pharmacological investigation of Myrica salicifolia extract.

a) Central Nervous System effects

When M. salicifolia extract was injected into mice and the animals evaluated on the observational schedules described by Turner (1965), Irwin (1972) and Malone (1977) the following effects were noted: sedation, suppression of spontaneous and locomotor activity, analgesia, muscle relaxant activity, catalepsy and palpebral ptosis. Sedation and locomotor suppression suggested that M. salicifolia extract is a Central Nervous System (CNS) depressant. That this sedation did not translate into loss of the righting reflex at high doses suggests that unlike the hypnotics or anaesthetics, M. salicifolia extract is a selective depressant. The combination of locomotor suppression, catalepsy and palpebral ptosis suggested that M. salicifolia extract possesses neuroleptic activity (Janssen and Van Bever, 1978).

The gross behavioural effects observed led to the subsequent detailed evaluation of M. salicifolia extract for locomotor, analgesic, muscle relaxant and neuroleptic effects.

The locomotor effects of M. salicifolia were studied more definitively on the open field apparatus. This device was introduced by Hall in 1934 to measure emotionality in rodents (Downing 1964). It is also a measure of the animal's excitation state, open field scores - ambulation, rearings and grooming bouts - being increased by central stimulants and decreased by depressants. M. salicifolia extract at all the doses tested (10-100mg/kg) decreased the scores in all the open field parameters. This is consistent with the classification of M. salicifolia extract as a CNS depressant: the depressants haloperidol and amitriptyline reduced open field scores while the stimulant d-amphetamine increased the scores.

The open field behaviour of mice treated with diazepam did not fit into this format. Diazepam (10mg/kg) produced noticeable ataxia in mice but instead of a decrease there was an increase in the open field scores. This is due to the effects of diazepam on the animal's emotional state. When a rat or mouse is placed in a strange environment for the first time it explores its surroundings carefully so the ambulation scores would be lower than in subsequent exposures to the same conditions (Ahtee and Shillito 1970).

Anxiolytics reduce the animal's apprehension and therefore increase its exploratory activity. This was what was observed with diazepam. This has also been reported elsewhere, with diazepam (Ahtee and Shillito 1970, Hirose et al 1981), chlordiazepoxide (Rushton et al 1973), nitrazepam, phenobarbitone and the benzodiazepinoxazole derivative CS-430 (Kamioka et al 1978). That M. salicifolia extract decreased rather than increased open field scores even at low doses (10 and 25mg/kg) which did not produce appreciable ataxia, suggests that the extract lacks anxiolytic activity.

As already noted, amphetamine signficantly increased the locomotor activity of mice in the open field apparatus. Neuroleptics inhibit this amphetamine effect and this has indeed been developed as a screening test for neuroleptic activity (Greenblatt et al 1980, Yamada and Furukawa 1980, Clark et al 1982). M. salicifolia extract was found to partially suppress amphetamine hypermotility. By comparison haloperidol completely antagonised amphetamine hypermotility. This suggests that M. salicifolia extract has some neuroleptic activity. Diazepam and amitriptyline which for comparative purposes were also tested on this model lacked neuroleptic activity.

M. salicifolia extract was inactive on the other screening tests for neuroleptic activity that were performed. These were the reduction of amphetamine stereotypy, apomorphine stereotypy and amphetamine lethality in aggregated mice. Haloperidol was active on all the tests. These results indicate that M. salicifolia lacks neuroleptic activity and that the result from the amphetamine hypermotility test is most probably a false positive. That is not without precedent.

Ethanol (which lacks neuroleptic activity) has activity on two of the neuroleptic models: it reduces the lethality of amphetamine in grouped mice and amphetamine hypermotility in mice (Spreux-Varoquaux and Simon 1980). It is possible that the reduction of amphetamine hypermotility seen with M. salicifolia extract is via the same (as yet unidentified) mechanism as ethanol rather than dopamine antagonism, the postulated mechanism of action of the neuroleptics (Barchas et al 1978, Clark et al 1982)

Although M. salicifolia extract did not protect aggregated mice from the lethal effects of amphetamine, it was observed that the extract pretreated mice were less excited and noisy than the control or even haloperidol pretreated mice.

This is especially notable since the enhancement of amphetamine lethality by aggregation has been attributed to excitement, fear and anxiety (Askew 1962) which neuroleptics presumably reduce.

Since M. salicifolia extract was inactive on the specific tests for neuroleptic activity it can only be assumed that palpebral ptosis and catalepsy produced by the extract in rats (where they were more apparent than in mice) are produced by mechanisms other than dopamine antagonism as in neuroleptics (Johnson et al 1979). Both parameters have actually been used in establishing potency of neuroleptics (Janssen and and Van Bever 1978).

It is worth pointing out that though antagonism of amphetamine and apomorphine stereotypy are widely used to screen for neuroleptic activity (Greenblatt et al 1978, 1980, Janssen and Van Bever 1978, Janssen et al 1965, Fowler et al 1977a, b) it has been suggested that they are actually screens for capacity to produce extrapyramidal side effects (a characteristic of most neuroleptics) rather than antipsychotic activity (Clark et al 1982, Greenblatt et al 1980).

Clozapine and thioridazine which unlike other

neuroleptics are inactive on these two models are unique in having very weak extrapyramidal effects in man (Barchas et al 1980). Further, thiethylperazine, a phenothiazine which is used as anti-emetic, antagonises apomorphine and amphetamine stereotypy and produces extrapyramidal effects but lacks neuroleptic activity (Barchas et al 1978, Greenblatt et al 1980). It is thought that neuroleptics produce their extrapyramidal effects and antagonism of apomorphine and amphetamine stereotypy of blocking dopamine receptors in the nigrostriatal pathway (Clark et al 1982) and their antipsychotic effects by blocking the mesolimbic pathway (Clark et al 1982, Johnson et al 1979).

While neuroleptics antagonise the effects of amphetamine, antidepressants have the reverse effect. Antidepressants enhance many of the effects of central stimulants like amphetamine and lysergic acid diethylamide (LSD) (Brucke et al 1969). These effects include motor hyperactivity and stereotypy (Halliwell et al 1964), conditioned avoidance behaviour (Carlton 1961, Scheckel and Boff 1964) and hyperthermia (Jori and Garattini 1965). Potentiation by tricyclic antidepressants of amphetamine stereotypy in rodents has been used for screening similar acting drugs (Fukushima et al 1977, Mashkovsky and Andrejeva 1981, Meignen et al 1982).

M. salicifolia extract produced an increase in the duration of amphetamine stereotypy in rats. Amitriptyline had the same effect though its effect was more pronounced. This suggests that M. salicifolia extract had some antidepressant activity. Antidepressants potentiate amphetamine by inhibiting its metabolism and probably by having direct effects on central adrenergic mechanisms. (Brucke et al 1969).

M. salicifolia extract was inactive on the next screening test for antidepressant that was used. Unlike amitriptyline, the extract, at the dose used, failed to antagonise reserpine-induced ptosis in mice. This is the most widely used antidepressant screening test (Fukushima et al 1977, Greenblatt et al 1978, Karasawa et al 1978, Mashkovsky and Andrejeva 1981, Jalfre et al 1982, Meignen et al 1982, and Poncelet et al 1984). The test is based on the finding that reserpine depletes monoamines and produces severe depression in humans (Brucke et al 1969, Barchas et al 1978) which is responsive to antidepressants (Brucke et al 1969). In addition, the reserpine induced 'depression' in rodents characterised by palpebral ptosis, hypomotility and hypothermia is also responsive to antidepressants (Brucke et al 1969, Gelenberg

and Klerman 1978). Tricyclic antidepressants antagonise reserpine by increasing the rate of noradrenaline synthesis and reducing noradrenaline reuptake hence delaying its depletion (Brucke et al 1969) (Lemoine et al [illegible]).

M. salicifolia extract was also inactive on the behavioural despair test in the mouse. This test for antidepressant activity is based on the concept of 'learned helplessness', that is, when an animal is placed in an aversive situation from which it cannot escape it soon becomes apathetic and stops trying to get out (Maier 1984). In this particular model a mouse is forced to swim in a restricted space from which it cannot escape. The mouse gradually assumes a characteristic immobile posture after vigorous attempts to swim out fail (Porsolt et al 1977, 1978). The duration of this immobility is reduced by a wide variety of clinically effective antidepressant treatments: tricyclic antidepressants, monoamine oxidase inhibitors (MAOI), the atypical antidepressants and even electroconvulsive shock (Porsolt et al 1977). The atypical antidepressants are a new group of drugs (for example mianserin) which have different biochemical and pharmacological properties from the 'classical' antidepressants, the tricyclics and MAOI's and which

are inactive on the usual animal screening tests (Porsolt et al 1977). The antidepressant activity of mianserin for example, was discovered from the quantitative evaluation of human EEG's (Itil 1978). The versatility of the method has already made it a very popular screening procedure (Karasawa et al 1980, Borsini et al 1981, Jalfre et al 1982, Poncelet et al 1984, Luttinger et al 1985).

The method is not without limitations however. It has been reported that it is not specific to antidepressants since anticholinergics and antihistamines also reduce the duration of immobility in the test animals (Nomura et al 1982).

The inactivity of M. salicifolia extract on two antidepressant screening tests casts doubts on the results from the amphetamine stereotypy test. This needs further evaluation but it can be considered that the extract lacked antidepressant activity.

M. salicifolia root extract showed potent analgesic activity on the hot plate test and the tail pressure test on uninflamed tissue. Only the centrally acting morphine - like analgesics show activity on these two models of pain, the peripherally acting analgesics like aspirin are inactive (Peterfalvi et al 1975, Winter 1965) with even large doses producing very small, uncertain

M. salicifolia extract failed to protect mice against convulsions induced by three different chemical convulsants:- 3-mercaptopropionic acid, bemegride and caffeine. By contrast diazepam at 10 and 20 mg/kg was effective against all the three chemoconvulsants. Since most clinically useful anticonvulsants, phenobarbitone, trimethadione, chlordiazepoxide, carbamazepine, ethosuximide and mephenytoin are effective against most chemical convulsants (Stone and Javid 1979, 1980) it may be concluded that M. salicifolia extract is not an anticonvulsant. Effectiveness against chemically-induced convulsions as a screening test for potential anticonvulsants however has some limitations - the widely used antiepileptic phenytoin sodium is inactive against convulsions induced by pentylenetetrazole, 3-mercaptopropionic acid, bemegride, bicuculline and picrotoxin (Stone and Javid 1979). It even potentiates convulsions due to imidazole and aminophylline (Stone and Javid 1980), and would therefore have been rejected on this screening model. With this is mind, though M. salicifolia extract was inactive against chemoconvulsions the assertion that it lacks anticonvulsant activity must await tests against electroshock convulsions.

M. salicifolia extract failed to protect mice from the lethal effects of physostigmine (1.0mg/kg). This indicates that the extract lacks central anticholinergic activity. Centrally but not peripherally acting anticholinergics antagonise physostigmine lethality in rodents. This test has been used to screen for antiparkinsonian drugs acting by blocking central cholinergic receptors (Nose and Kojima 1970). Some hypnotic or anticonvulsant drugs like the barbiturates and benzodiazepines however protect rodents against physostigmine lethality although they lack anticholinergic effects (Niemegeers et al 1982).

M. salicifolia produced impairment of motor performance in mice and rats. Since the extract did not affect the neuromuscular junction it follows that this effect is not due to muscle paralysis but rather to interruption of afferent pathways. Production of ataxia is a characteristic of CNS depressants in high doses and is consistent with the central depressant effects of the extract.

M. salicifolia extract prolonged the duration of ether anaesthesia in rats and ethanol and pentobarbitone hypnosis in mice.

Since these three compounds are metabolised by different pathways it is unlikely that M. salicifolia potentiates their hypnotic effects merely by decreasing the rate of their metabolism. It may be that M. salicifolia extract acts by sensitising the system to the action of anaesthetic agents or by interrupting the afferent pathways (Dandiya and Sharma 1962).

Lessin and Parkes (1957) have shown that the reduction of locomotor activity and prolongation of pentobarbitone hypnosis in mice by chlorpromazine and reserpine is proportional to the fall in body temperature produced by these drugs. Further Fastier et al (1957) have shown that the prolongation of chloral hydrate hypnosis in mice by adrenaline, serotonin, histamine, atropine, yohimbine, tolazoline and ergotamine is a factor of their hypothermic effect. M. salicifolia extract (100mg/kg) produced a significant fall in the normal body temperature of rats and it is most probable that the extract potentiated the hypnotic effect of ether, ethanol and pentobarbitone by causing hypothermia. Although non-hypnotic on its own, the sedative effects of the extract would also be expected to augment the hypnotic effect of the other CNS depressants hence extending the duration of their anaesthetic effects.

M. salicifolia extract (at 100m/kg) exhibited potent antipyretic activity when tested against fever induced in rats by subcutaneous injection of brewer's yeast. However, unlike, acetylsalicylic acid, the extract produced hypothermic activity indicating that the activity in fevered rats may be hypothermic rather than true antipyretic activity. In the acetylsalicylic acid treated pyretic rats, the temperature was brought down to normal levels while in M. salicifolia treated group, the temperature fell well below the normal range. This hypothermic effect of M. salicifolia extract was most apparent when the extract is given to non-pyretic rats when the rectal temperature fell below $35^{0}C$, the lowest measurable with a clinical thermometer. In the acetylsalicylic acid treated group there was no difference with the controls. The hypothermic effect of M. salicifolia was probably due to the central depression it produces though peripheral mechanisms may also be involved.

It is worth considering whether M. salicifolia extract has anxiolytic effects. Anxiolytics, according to the World Health Organisation (WHO) definition "are substances which reduce pathological anxiety, tension and agitation without therapeutic effects on cognitive or perceptual processes" (Schallek 1978).

Prelinical screening for anxiolytic activity involves a battery of behavioural tests: taming behaviour such as reduction in electroshock - induced fighting in mice; muscle relaxant activity in mice or rats; anticonvulsant activity against chemical or electroshock convulsions; effect on conditioned avoidance response in rats or squirrel monkeys; and attenuation of conflict-induced suppression of behaviour in rats: a conflict situation being the simultaneous presentation of a reward (food) and a punishment (electric shock) (Randall and Kappel 1973, Kamioka et al 1978, Petracek et al 1978, Schallek 1978, Pieri et al 1981). Potency in this battery of tests was found to be correlated to clinical efficacy and was used to establish structure-activity relationship of the major class of anxiolytics, the benzodiazepines (Sternbach et al 1968). The activity of M. salicifolia extract on two of these tests was used in determining whether it has anxiolytic activity.

M. salicifolia extract produced muscle relaxant activity. This however is also an indicator of sedation and is common to the central depressants. The extract had no effects on chemoconvulsions produced by a range of convulsants. This indicates that it is unlikely to have anxiolytic properties. This conclusion is supported by the activity profile on open field behaviour in the mouse.

The benzodiazepine, diazepam increased open field activity in the mouse - an effect that can conceivably be ascribed to its anxiolytic activity - while M. salicifolia extract reduced it.

b) Systemic Effects of M. salicifolia extract

M. salicifolia extract produced contraction in the guinea pig ileum. These contractions were antagonised by atropine but not by hexamethonium or mepyramine. This means that M. salicifolia extract has muscarinic effects since atropine selèctively blocks effects produced by stimulation of muscarinic cholinergic receptors. Resistance to hexamethonium or mepyramine blockade shows that the effects of the extract are respectively neither nicotinic nor histaminic.

The extract also increased the tone and amplitude of contractions of the rabbit jejunum.

Given orally M. salicifolia extract had no effect on gut motility. On intraperitoneal administration however the extract produced drastic reduction in gut motility as indicated in the reduction in the distance moved through the small intestine by a charcoal meal administered orally by gavage. This is inconsistent with the effect observed on the

isolated gut, which would have led one to expect diarrhoea. It would appear that on intraperitoneal administration the extract is absorbed and undergoes biotransformation to give metabolites that are inhibitory to intestinal movements. Alternatively, irritation of the peritoneum by the extract might provoke a nervous reflex whose effect is to decrease the intestinal movements. A third possible explanation is that in producing the peritonitis observed when the extract is administered intraperitoneally some fluid is drawn from the intestines. Drawing water from gut would drastically decrease intestinal movement, just as the reverse effect, water flux into the intestines produces diarrhoea.

Administered orally to 24 hour fasted rats M. salicifolia extract produced corrosion of the gastric mucosa. This ulcerogenic effect was also shared by the antiinflammatory and gastric irritant drug, indomethacin and is probably a reflection of the irritant activity of the extract. This irritant effect is also indicated by the writhing response observed when the extract is administered intraperitoneally in rats and mice. The accumulation of large volumes of liquid in the peritoneal cavity of rats and mice injected with the extract intraperitoneally also points to an irritant effect.

This ulcerogenic liability indicates that the extract should not be taken on an empty stomach; when used traditionally to produce psychotropic effects it was not.

The effects of M. salicifolia extract on the cardiovascular system appear to be inconsistent. The extract produced a fall in the blood pressure of the anaesthetised rat, a rise in the perfusion pressure of the rat hindquarters and no effect in the perfused isolated heart. The lack of effect on the isolated heart indicates that the extract is not cholinergic, histaminic or adrenergic. The rise in the perfusion pressure of the rat hindquarters indicates increased peripheral resistance suggesting that M. salicifolia extract caused release of noradrenaline, peripherally.

From these two sets of results a rise in blood pressure in the intact rat would be expected. The blood pressure fall that actually occurs persists even when the rat is atropinised indicating that it is not a cholinergic effect. It is most likely to be a central rather a peripheral effect. M. salicifolia extract potentiated the central anaesthetic effects of ether, pentobarbitone and ethanol and would be expected to potentiate the effects of urethane, the anaesthetic used in this experiment. The fall in blood pressure, heart rate and respiratory rate observed on injecting

the extract, effects which become more marked on repeated injection of the extract, is consistent with potentiation of urethane anaesthesia.

Administered orally, M. salicifolia extract had no effects on urine output. However, when injected intraperitoneally the extract was a potent antidiuretic, with 100mg/kg body weight completely stopping urine output over the six hours the rats were observed. On killing the rats and opening the abdomen, the bladder was found empty but a lot of liquid had accumulated in the peritoneal cavity. The extract draws water from the rest of the body to the peritoneum - for instance the blood was found to be very viscous presumably because of efflux of fluid to the peritoneum. The antidiuretic effect is therefore probably due to the massive peritonitis leaving no liquid to be filtered by the kidneys.

M. salicifolia extract was inactive against carregeenan-induced paw edema in the rat. Carrageenan edema is an acute and exudative manifestation of inflammation and is unlike clinical human arthritis which is a chronic and proliferative condition. It is however the most widely used antiinflammatory screening method (Carrano et al 1975, Caliari et al 1977 Shibata 1977, Sofia et al 1977a, Garcia Rafanell et al 1980) because it has a short latent period (2-4 hours)

and is responsive to most clinically active agents (Paulus and Whitehouse 1972). Animal models which involve the proliferative response such as cotton pellet granuloma in rats (Caliari et al 1977, Petit et al 1977, Shibata 1977) resemble arthritis more, but are less suitable for screening purposes as they do not respond to some drugs e.g. the salicylates. The lack of activity on the carrageenan test therefore suggests that M. salicifolia extract is devoid of antiinflammatory activity.

M. salicifolia extract had neither local anaesthetic activity nor effects on the neuromuscular junction. This means that the paralysis of the hind limbs observed in some rats and mice is most likely a central rather than a local effect on the peripheral nerves or skeletal muscle.

On the guinea pig trachea M. salicifolia extract produced contractions which were antagonised by atropine sulfate. This means that the contractions were produced by stimulation of muscarinic receptors. This muscarinic effect was also observed on the rat uterus, where like acetylcholine contractions, those due to M. salicifolia were abolished by atropine sulfate.

It is concluded therefore that the uterine effect of the extract was cholinergic rather than oxytocic.

It is worth noting that the muscarinic effects the extract exhibits on smooth muscle (the uterus, trachea, ileum and jejunum) were not observable on the isolated rabbit heart. Further work would be necessary to explain this odd situation.

With a median lethal dose (LD_{50}) of 248 ± 22mg/kg *M. salicifolia* extract can be considered to be only moderately toxic. The maximum working dose for animal experiments should not be above 125mg/kg (approximately half the LD_{50}) as the number of deaths within 24 hours was appreciable.

Chapter Seven

CONCLUSION

M. salicifolia root extract produced a variety of central nervous system effects in rats and mice. These include sedation, palpebral ptosis, inhibition of locomotor activity, ataxia, catalepsy, hypothermia, centrally - mediated analgesia, antipyretic effects and potentiation of the hypnotic effect of CNS depressants.

M. salicifolia extract lacked neuroleptic, antidepressant, anxiolytic and anticonvulsant effects. Yet the effects the extract is said to produce in humans, which include emotional incontinence, aggressiveness, feelings of invincibility and overaction to external sound could indicate that it possesses psychotropic effects.

These human psychotropic effects fit into Downing's definition of a psychotomimetic agent as 'one which will consistently produce changes in thought, perception, and mood, occuring alone or in concert, without causing major disturbances of the autonomous nervous system or other serious disability' (Downing 1964). Such effects are by their nature exclusively human effects which are if not impossible difficult to demonstrate in animals.

It is difficult to find factors in a rats behavioural repertoire which correspond to alterations in perception and thought patterns.

Hence, for instance extracts of Psilocybe mexicana produce no discernable effects in mice and dogs but produce marked psychotomimetic effects when ingested by man (Hofmann 1968). Mescaline produces only ataxia and motor paralysis in mice (Hofmann 1968) yet produces dramatic hallucinations in humans (Huxley 1956). The remarkable effects of lysergic acid diethylamide (LSD) in man could not have been predicted from the excitation observed in some of the rats given the drug (Hofmann 1968).

Despite these reservations however psychotomimetics are amenable to investigation in animal models. Two approaches are worthy of note.

These are conditioning experiments using shuttle - boxes or Skinnerian cages where the effect of the drug on performance of a task in which a rat has been trained is investigated; and qualitative evaluation of animal EEG's. The former has been shown to detect the psychotomimetic effects of mescaline (Smythies 1968) and the glycolate esters (Abood 1968) while the later those of LSD, mescaline, psilocybin and the tetrahydrocannabinols (Longo 1978) in animals.

Both approaches are proposed in further work to establish the pharmacological basis of the effects of M. salicifolia extract.

Although human experimentation would have yielded interesting confirmatory evidence this was not done. For one the toxic effects observed in animals, such as hindlimb paralysis raise the spectre of this happening in man although it has not been reported. The other fear was that the psychotropic effects of the extract are unpleasant and are reputed to persist well after its use had stopped. This however does not preclude experiments with humans if proper precautions are taken.

In the absence of independent confirmation of human effects of M. salicifolia extract, the descriptions obtained from interviews with ex-morans and which gave impetus to the present work must be viewed with caution. Drug effects in animals being difficult to extrapolate to humans, the results obtained in this work can neither be said to refute or confirm the accounts of the former morans. The work has however made an important contribution in that there now exists a pharmacological profile of M. salicifolia extract which had not been documented before and which will provide an important basis for more detailed work on this medicinal plant in future.