PART - D

PHARMACOLOGICAL STUDIES

ON THE ESSENTIAL OIL OF

XANTHOXYLUM ALATUM AND

ITS ACTIVE PRINCIPLES
INTRODUCTION

Biological activity of the terpenoids and their derivatives

The terpenoids constitute one of the largest groups of naturally-occuring compounds, being characterized by their extremely widespread distribution in both the plant and animal kingdom, and by the great diversity of their chemical structures (De Mayo, 1959; Simonsen, 1949, Barton, 1953)\(^{126-128}\). Extensive biological investigations have been carried out within the group and these studies have revealed a broad spectrum of pharmacological and physiological properties, some of which have led to a number of terpenoids gaining medicinal application. Few of the simpler naturally-occuring terpenoids are in chemical use today. The recent demonstration that certain nitrogenous terpene derivatives possess potent anti-hypertensive activity may indicate that a new era in medicine - that of the synthetic terpenoids - is about to dawn. The anti-microbial and insecticidal properties of other terpenoids have led to their utilization as pesticides and fungicides in agriculture and horticulture.

The majority of monoterpenoids are obtained from the essential oils of plants in which they occur in admixture with volatile compounds of other chemical groups (Guenther, 1949)\(^{129}\) and at one time the essential oils saw considerable medicinal application; often without any true rationale. The "Dispensatorium Valeri Cordi" of 1592 (Smith and Khatoon, 1963)\(^{130}\) listed some 60 different essential oils, but their lack of importance in modern medicine is indicated by the fact that a
recent text-book on therapeutics (Current Therapy, 1959)\textsuperscript{131} makes only one or two minor references to monoterpenoids as constituents of lotions. The main medicinal use of the essential oils and their constituent monoterpenoids today is as carminatives, although recent claims that certain essential oils prevent the development of atherosclerotic plaques (Benco et al., 1961)\textsuperscript{132} stimulate cellular defense mechanisms (Smith and Khatoon, 1963)\textsuperscript{130}, may well lead to a reinvestigation of their potentialities. Citral has been claimed to be of benefit in preventing alimentary atherosclerosis in rabbits but as this monoterpenoid in common with several others is known to produce fatty liver infiltrations in mammals, it would seem unwise to pin high hopes upon such terpenoids as possible clinically desirable prophylactic agents against atherosclerosis.

At one time various essential oils and their constituent terpenoids saw application in combatting infections particularly those of the bronchial and urinary tracts (Winter, 1958)\textsuperscript{133} and in preventing the sepsis of burns and wounds. With the advent of sulphonamides and the antibiotics, terpenoids are seldom used for such purposes today; although it should be noted that the potential anti-bacterial properties of hinokitol (\(\beta\)-thujaplicin) (Okazuki and Homma, 1954)\textsuperscript{134} may secure for this compound a limited clinical application. Not only is hinokitol claimed in some instances to exhibit a higher potency than the common antibiotics (Smith and Khatoon, 1963)\textsuperscript{130} it is also been claimed that bacteria do not readily develop strains resistant to this agent (Smith and Khatoon, 1963)\textsuperscript{130}.

On the other hand terpenoids still find extensive application
as disinfectants of objects likely to harbour microorganisms and as air-freshening disinfectants sprays. The antiseptic properties of essential oils (Code, 1957; Profand 1960)\textsuperscript{135,136} and the pine oil disinfectants (Smith and Khatoon, 1963)\textsuperscript{130} have been reviewed. Thymol and carvacrol are still used extensively in mouthwashes, and various monoterpenoids are incorporated in toothpastes and oil of clove, which contains the

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\begin{align*}
\text{Thymol} & \quad \text{Carvacrol} \\
\begin{array}{c}
\text{OH} \\
\text{OH}
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\end{align*}
\]

sesquiterpene caryophyllene which is still used extensively in dentistry.

Many of the simpler terpenoids are characterized by the possession of irritant properties and certain essential oils such as oil of Juniper, were once used as diuretics because of the irritation produced in the kidneys.

Externally certain essential oils may still be used as counter-irritants and rubifacients in the form of embrocations and liniments. They produce an initial feeling of warmth and smarting which is often followed by a mild local anaesthesia, making them of value in anti-pruritic preparations. Menthol, camphor, guaiacol and terpin hydrate find applications as cough stimulants (Smith and Khatoon, 1963)\textsuperscript{130}.

Particularly pronounced irritant properties are present in canthardin as indicated by its ability to produce vescication even
in the skin of corpses and recently a renewed interest has been shown in the biochemical mechanism of cantharidin acantholysis. But it has toxic effects on absorption. There are reports that cantharidin can successfully inhibit tumour induction by carcinogenic tar (Berenblum, 1935)\(^1\)\(^3\)\(^7\) an action which may also be attributable to its irritant properties which result in a sloughing of the embryonic tumour. There is also a report of a successful clinical cure of a cancerous growth (Smith and Khatoon, 1963)\(^1\)\(^3\)\(^0\). Carcinostatic properties have been reported for a variety of other terpene oils including citral, citronellal and S-guaiazulene (Smith and Khatoon, 1963)\(^1\)\(^3\)\(^0\).

but such compounds do not appear to possess truly significant activity.

Although irritant activity may be said to be one of the most characteristic properties of the simpler terpenoids, there are a number of claims that certain essential oils and their constituent terpenoids
possess anti-inflammatory properties. One oil subject to such claims was camomile oil (Smith and Khatoon, 1963; Pommer, 1942)\textsuperscript{130,138} and the subsequent isolation of chamazulene from this oil gave rise to an extensive investigation of sesquiterpenoid azulenic hydrocarbons as anti-inflammatory agents (Janitsyn, 1951)\textsuperscript{139}.

There have been a number of claims in the Russian literature (Rokhлина et al., 1948; Bakhovski and Provolovich, 1957)\textsuperscript{140,141} that certain monoterpenoids such as citral, geraniol, allocimene and the ionones possess antihistaminic activity and the statement has been made that citral is widely used by Russian ophthalmologists as an anti-inflammatory agent.

Several terpenoids such as thymol, alantolactone, 1-\(\alpha\)-santonin etc. have clinical use as anthelmintic agents. Camphor was once widely used in clinical practice, having been introduced into Western medicine, without any rationale, in mediaeval times after a long history of use in ancient Chinese remedies. But it has been clearly shown that camphor does not possess antipyretic activity of cardiovascular and respiratory stimulant properties (Smith and Khatoon, 1963)\textsuperscript{130}.

Picrotoxin, which is a molecular compound of the sesquiterpenoid picrotoxinin and its hydroxylated derivative, picrotin, formerly saw considerable application in clinical medicine on account of its ability
to counteract the central depressant effects of barbiturate intoxication (Maloney, 1941; Bleckwenn et al., 1937; Report of the Council on Pharmacy and Chemistry 1949; Koppanyi and Fazakas, 1950)\textsuperscript{142-145}. This use was not without danger (Eckenhoff, et al., 1949)\textsuperscript{146} and the drug has now been replaced by amphetamine and \(\beta\)-methyl glutarimide. Picrotoxin also saw limited trial in the convulsion treatment of schizophrenia.

One group of highly oxygenated bitter principles, the curcurbitacins are cytoxins and are currently being investigated for antitumour activity (Gitter et al., 1961)\textsuperscript{147}.

Despite the steady displacement from clinical medicine of such terpenoids as those just discussed, there are several indications that the full clinical potentialities of the terpenoids have not yet been realized. Thus in his review of the cardiac properties of the \textit{Erythrophleum} alkaloids McCawley (1955)\textsuperscript{148} points out that if these compounds could be obtained cheaply, they might hold promise as digitalis substitutes and indeed crude preparations of the alkaloids have seen clinical application (Smith and Khatoon, 1963)\textsuperscript{130}. Again the demonstration that a number of terpenoids, notably those related to glycyrrhetinic acid

\[
\text{Glycyrrhetinic acid}
\]
have the ability to interfere with steroid metabolism, (Atherden, 1958) resulting in a prolongation of action of the adrenocorticoid hormones, indicates that terpenoids may have a role to play as adjuncts to steroid therapy.

The synthetic terpenoids that have shown the greatest clinical promise so far, are certain nitrogenous monoterpenoid derivatives which exhibit hypotensive properties by virtue of their ability to block sympathetic ganglia. The best known of these agents is 3-methylaminoisocamphene hydrochloride (syn. mecaminine) which is characterized by its high potency and long duration of action, (Stone et al., 1956; Zawoiski, 1958) although, like all ganglion blocking agents so far tested, it does not show complete specificity for sympathetic ganglia and consequently it produces the usual undesirable side effects attendant upon blockade of the parasympathetic ganglia. Mecaminine does possess a considerable advantage over most other ganglionic blocking agents as it is active on oral administration unlike the ganglionic blocking agents possessing quaternary ammonium functions which are poorly and irregularly absorbed by the route. Extensive investigations are being continued in the aminoterpenoid field at the present time (Smith and Khatoon, 1963) and it does not seem unreasonable to anticipate that among new nitrogenous terpenoids will be found a number of clinically useful antihypertensive agents.

Other applications of biologically-active terpenoids

Insecticidal properties are exhibited by a number of terpenoids of widely diverse chemical constitution and some of these compounds
have at one time or another achieved considerable economic importance. Of the naturally-occurring insecticidal terpenoids (Smith and Khatoon, 1963)\textsuperscript{130} the most important are the pyrethrins I and II and the cinerins I and II, which occur in the flower heads of Chrysanthemum cinerariaefolium (Smith and Khatoon, 1963)\textsuperscript{130}.

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\begin{align*}
R = &-\text{CH}_2\text{CH}^\text{cis} = \text{CHCH} = \text{CH}_2 & R' = &-\text{CH}_3 & \text{Pyrethrin I} \\
R = &-\text{CH}_2\text{CH}^\text{cis} = \text{CHCH} = \text{CH}_2 & R' = &-\text{COOCH}_3 & \text{Pyrethrin II} \\
R = &-\text{CH}_2\text{CH}^\text{cis} = \text{CHCH}_3 & R' = &-\text{CH}_3 & \text{Cinerin I} \\
R = &-\text{CH}_2\text{CH}^\text{cis} = \text{CHCH}_3 & R' = &-\text{COOCH}_3 & \text{Cinerin II}
\end{align*}
\]

These compounds, which are esters of the monoterpenoid acids like, chrysanthenic acid and chrysanthemum dicarboxylic acid, are characterized by a low toxicity to warm blooded animals and inability to achieve quick knock down of insects. The coexistence of these desirable properties has inspired the preparation and screening of a number of synthetic esters of these acids in attempts to secure even more potent agents.

As a result of an extensive investigation, insecticidal properties have been shown to be present in various terpenoid derivatives,
particularly certain ethers, esters, halogenated compounds and sulphur derivatives (Smith and Khatoon, 1963)\textsuperscript{130}.

Several monoterpenoids have insect-repellent properties. Citronellal enjoyed a reputation as a mosquito-repellent before the introduction of synthetic agents.

On the other hand a number of monoterpenoids possess a pronounced attraction for certain insects and it is probable that the combination of attractant and repellent properties of essential oils play a role of some importance in the economy of the vegetable kingdom, just as their mild antibacterial and antifungal properties serve to protect the plants against noxious bacteria and fungi. Attraction towards certain insects would favour pollination, while repulsion would serve to protect against other harmful species.

Many essential oils and their constituents monoterpenoids are characterized by their agreeable odours and their palatable flavours, thus are of importance in the perfumery and cosmetic industries and as flavouring agents (Bedoukian, 1959; Molecular Structure and Organoleptic Quality, 1957; Pouchar, 1950)\textsuperscript{152-154}.

**Theoretical considerations**

Since the terpenoids display a wide range of biological activities, they are classed as structurally nonspecific drugs. Apart from the antimicrobial and irritant properties, which are responsible for their former medicinal applications, these compounds exhibit a spectrum of activities which includes haemolysis (Smith and Khatoon, 1963)\textsuperscript{130} met-haemoglobin production (Dessemontet, 1927)\textsuperscript{155}, inhibition of serum
cholinesterase (Drake and Stuhr, 1935)\textsuperscript{156}, spasmolytic activity (Zita et al., 1958; Van Liere and Northup, 1942, Hildbrandt, 1901)\textsuperscript{157-159} and paralysis of skeletal muscle. Often the pharmacological actions of these compounds are characterized by stimulation at low dosage levels, followed by depression at higher dosages as has been observed with respect to their actions on the gastro-intestinal tract, the central nervous system and the heart.

A more detailed illustration of the wide range of activities shown by the structurally nonspecific terpenoids is given by a consideration of the biological properties of camphor. Camphor has been shown to lower blood viscosity, to affect erythrocytes (Lipschitz, 1930)\textsuperscript{160} and to possess antihaemorrhagic properties, to depress isolated intestine and to produce an initial excitation followed by a depression of skeletal muscle; it has also been claimed to check insulin convulsions (Smith and Khatoon, 1963)\textsuperscript{130} and to exert direct and reflex actions on the central nervous system (Drake et al., 1956)\textsuperscript{161}.

Structural nonspecificity would appear to extend into the triterpenoids, but structural specificity becomes noticeably apparent at the sesquiterpene level. Some of them show antimicrobial activity and these presumably play an analogous role in nature to that of the lower members in protecting the plant against harmful bacteria and fungi.

**Terpenoids as structurally specific agents**

Apart from the rethrins, in which small changes in stereochemistry may lead to striking changes in insecticidal potency and the synthetic ganglion blocking agents, structural specificity does not
seem to occur in monoterpenoids (Smith and Khatoon, 1963)\textsuperscript{130}.

Drug latentiation studies

Terpenoids have played a small but nevertheless significant role in drug latentiation (Harper, 1959)\textsuperscript{162} where a chemical derivative of an active drug is administered in order to overcome unfavourable rates of biotransformation, or unfavourable solubility distribution, transport, chemical stabilization of drug molecules etc. These include the preparation of sparingly-soluble aminoterpenoid penicillin salts capable of maintaining prolonged therapeutic concentrations of the antibiotic in the blood stream.

The ethanolamine system and its simple N-alkylated derivatives appear as the active radicals of many antispasmodic, local anaesthetic, sympathomimetic, and antihistaminic drugs, and a considerable number of synthetic terpenoids incorporating substituted \( \beta \)-ethanolamine moieties have been subject to pharmacological study. Prominent among such compounds are a number of ethers. Thus 3 (\( \beta \)-diethylaminoethoxy-\( \beta \)-cymene possesses spasmylytic antihistaminic and hypnotic properties (Smith and Khatoon, 1963)\textsuperscript{130}, antispasmodic properties are also present in the corresponding \( \beta \)-dimethylaminoethyl ether and in menthyl-\( \beta \)-dimethylaminoethyl ether (Reinhard \textit{et al.}, 1951; Grail \textit{et al.}, 1952)\textsuperscript{163,164}. Sedative properties are also exhibited by certain of these terpenoid ethers and a number of compounds of the type shown have been carefully
investigated for their ability to prolong barbiturated narcoses and to afford protection against electroshock and metrazole shock (Smith and Khatoon, 1963)\textsuperscript{130}. Several derivatives of 2-amino-1-p-menthanol synthesized from d-limonene were without physiological activity, although they exhibited antifungal properties as does 2-diethylaminoethyl fencholate (Tilford et al., 1949)\textsuperscript{165}.

Local anaesthetic properties were found to be present in menthyl aminoacetate and 2-benzoyloxy-5-diethylaminoborane while the hydrochloride of the β-diethylaminoethyl ester of cumic acid has been claimed to be as potent a local anaesthetic as procaine hydrochloride (Williams and Voss, 1951)\textsuperscript{166}.

The bornane skeleton has seen considerable utilization as a supporting moiety. For example, two isomeric 2-hydroxy-3methyl-aminobornanes have been shown to have a pronounced action on the respiratory center of rabbits (Smith and Khatoon, 1963)\textsuperscript{130} and 2, 3-dihydroxybornane and its monocarbamate have been demonstrated to be central nervous system depressants.

Derivatives of the type that can be regarded as analogues of succinylcholine formed by replacement of the succinic acid moiety by the camphoric acid moiety were found to be active as
neuromuscular blocking agents (Schilling and Pedersen, 1956)\textsuperscript{167}.

The discovery that marked antituberculous properties were present in various thiosemicarbazones, hydrazones and 4, 4'-diaminodiphenyl-sulphone derivatives resulted in the synthesis of a large number of compounds belonging to these groups including terpenoid derivatives in which the terpenoid portion can be regarded as a supporting moiety.

Further examples of utilization of terpenoids as supporting moieties which may be quoted are, the preparation of the geranyl, citronellyl, and linaloyl esters of chaulmoogric acid in attempts to increase the efficacy of chaulmoogra therapy for leprosy, the synthesis of the menthyl esters of cinchopen and the bornyl ester of salicylic acid in attempts to increase the potency of the parent compound and the preparation of terpenyl substituted fatty acids as potential antibacterial agents (Smith and Khatoo, 1963)\textsuperscript{130}.

The rigid nucleus of certain diterpenoids acts as an alternative supporting moiety for some oestrogenic steroids. These include 6-hydroxydehydroabietinol and its C-4 epimer, 7-isopropyl podocarpinol, podocarpinol, etc. (Baizer et al., 1950)\textsuperscript{168}.  

107
Utilization of a terpene nucleus as a supporting moiety has also been reported in connection with attempts to prepare potential morphine-like analgesics (Fieser and Campbell, 1939)\textsuperscript{169}.

**Biotransformation**

Terpenoids have played an important role in studies of the biotransformation and metabolism of compounds foreign to the body, but as many compounds can be considered to be normal constituents of animal food, the ability of the animal organism to metabolize them into compounds more easily excreted and eliminated has not had as great an impact on scientific thinking as has the ability of animals to effect biotransformation of the newer synthetic compounds (Smith and Khatoon, 1963)\textsuperscript{130}.

The successful development of methods involving microbiological transformations of steroids for the preparation of new medicinal agents has served to inspire studies on the microbiological transformations of terpenoids (Bhattacharyya et al., 1963)\textsuperscript{170}. It can be assumed that these transformations, will lead to new roles for terpenoids in drug synthesis (De Mayo and Reid, 1961)\textsuperscript{171}. 

\[ \text{6-Hydroxydehydroabietinol} \quad \text{Podocarpinol} \]
MATERIALS AND METHODS

Male albino rats (Sprague-Dawley 160-210 g) were used in all the experiments except those for conditioned avoidance response for which male albino rats (Haffkine strain, Haffkine Institute, Bombay) were used. In experiments using mice, male albino Swiss mice (23-30 g) were employed. All the experiments were performed at a room temperature of 23±1°C.

The essential oil of *Xanthoxylum alatum* and its active constituents linalool and methyl cinnamate were prepared as a fine emulsion in 3 per cent polysorbate-80 (Tween-80) solution. Methyl cinnamate was dissolved in a minimum quantity of absolute alcohol before the addition of Tween 80, the alcohol content of the final solution being 10 percent. All the other drugs were dissolved in distilled water. The volumes of these solutions were such that 0.4 ml contained the dose required per 100 g body weight of the animal. Injections were always made intraperitoneally. Control experiments using the appropriate solvents were performed simultaneously.

*Drugs used in this study and their sources are given below:

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>Supplier</th>
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<tbody>
<tr>
<td>Pentobarbitone sodium (Nembutal sodium)</td>
<td>Winthrop Laboratories, New York</td>
</tr>
<tr>
<td>Hexobarbitone sodium (Evipal sodium)</td>
<td>&quot;</td>
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<tr>
<td>Morphine hydrochloride</td>
<td>U.S.V. Laboratories, Washington</td>
</tr>
<tr>
<td>Chlorpromazine hydrochloride</td>
<td>Smith, Kline and French Labs., Philadelphia</td>
</tr>
<tr>
<td>d-amphetamine sulfate</td>
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</tbody>
</table>
Spontaneous motor activity of mice

A total number of 60 mice were used for these experiments. For each dose level, three experiments were performed on three different days using 5 mice for each experiment. The essential oil suspension was administered in doses of 70, 140 and 210 mg/kg body weight and the control animals were treated similarly with the solvent. Since a dose of 350 mg/kg was found to cause marked depression leading to hypnosis in a majority of the animals, doses higher than 210 mg/kg were not used for these studies.

After the drug or solvent treatment, the animals (n = 5) were kept together in a plastic cage (28 cm x 19 cm x 12 cm) which was placed over an activity meter (Selective Activity Meter, Model 25, Columbus Instruments Co. Columbus, Ohio) and the locomotor activity of the animals was recorded for 2 hours.

Determination of the hypnotic dose of the essential oil and its comparison with long- and short-acting barbiturates

Groups of mice (n = 10) and rats (n = 10) were used for each experiment. The essential oil was administered in doses of 300, 350, 400 and 450 mg/kg to different groups of animals and the number of animals losing their righting reflex (those animals unable to regain their normal posture when placed on their back), and the duration of the hypnotic effect were noted for each animal. Sleeping time is the time elapsed between the loss of righting reflex and the time at which the animals regained it.

For comparative purposes, the short-acting barbiturate pento-
barbitone sodium (50 mg/kg) and the ultra-short acting barbiturate hexobarbitone sodium (100 mg/kg) were administered to other groups of mice and their hypnotic effects were compared with those of the essential oil.

Investigation of the hypnotic effects of the major constituents of the oil

The chemical analysis of the essential oil of *Xanthoxylum alatum* showed that its major constituents are methyl cinnamate (30 per cent) and linalool (22 per cent), one or both of which might be responsible for the major pharmacological effects of this oil on experimental animals. In order to determine this, the hypnotic effects of the oil were compared with those produced by the above constituents, individually and in a mixture.

Forty mice were used for this experiment. They were divided into four equal groups and different groups were treated with 1) essential oil (400 mg/kg), 2) linalool (88 mg/kg), 3) methyl cinnamate (120 mg/kg) and 4) a mixture containing linalool (88 mg/kg) and methyl cinnamate (120 mg/kg). The animals were placed in individual cages and the number of animals showing hypnotic effect and the duration of sleep were noted as before.

Effects of sub-hypnotic doses of the essential oil on the pentobarbitone-induced hypnosis in mice

Groups of 10 mice were used for each experiment. Three groups of animals were pretreated with the essential oil (70, 140 and 210 mg/kg), whereas the fourth group received the solvent. Five minutes later all the
groups received pentobarbitone sodium (50 mg/kg) and the sleeping time of individual animals was noted as before.

Rectal temperature of mice

Thirty mice were used for each experiment. The animals were divided into three equal groups, their core rectal temperature was noted and two of these groups were treated with the essential oil in doses of 210 and 400 mg/kg respectively. The solvent was administered to the third group. The rectal temperature was again noted at 5, 15, 30, 60 and 120 min after drug or solvent injections.

For recording the rectal temperature a telethermometer (Model 44TA Yellow Springs Instruments Co., Ohio) was used and the thermistor probe was introduced 4 cm into the rectum.

Analgesic effect of the essential oil

Influence of the essential oil on the pain sensation of rats was measured using an analgesiometer. The equipment used for this purpose was based on the principle of the 'hot-wire'analgesiometer' described by (Davis et al, 1946)\textsuperscript{172}. The author is grateful to Dr. Eric Pfeiffer, Ph.D., Western Research Center, Veteran Administration Hospital, Sepulveda, California for constructing this equipment. A bulb (100 W) was the heat source and a glass slide covered the upper surface of the bulb over which the tail of the animal could be placed. A small exhaust fan placed underneath the bulb efficiently prevented the equipment from getting too warm. An electric timer and the heat source could be operated simultaneously by pressing a switch.
In practice, the animal was placed in a restrainer with the tail hanging out. The tail (2 cm from the tip) was placed over the glass slide and the bulb was switched on. This automatically turned on the timer too. When the animal feels pain, it flicks the tail and the time taken to obtain the flick response was noted for each animal. This value ("reaction time") was found to be remarkably constant in randomly selected rats and mice. A drug which decreases pain sensation, naturally would prolong the reaction time.

Fifty rats were used for this experiment. The animals were divided into four equal groups, the reaction time of each animal was noted and different groups received the following treatment, namely 1) solvent, 2) essential oil 70 mg/kg, 3) essential oil 140 mg/kg, 4) essential oil 210 mg/kg, and 5) morphine hydrochloride (5 mg/kg). Morphine hydrochloride was used as a reference standard with which the analgesic effects of the essential oil could be compared. After drug or solvent treatments, the reaction time was again noted at 15, 60 and 120 min.

Studies on the local anesthetic effects of the essential oil

Eighty mice were used for this experiment. The method employed for the evaluation of the local anesthetic effects was based on the one described by Bianchi (1956)\textsuperscript{173}, the only modification made being that the pain sensation over the tail was evaluated by employing the analgesiometer in place of the artery clip employed by the above workers.

The principle of the method is that if a local anesthetic is injected at or near a nerve, pain applied to the organ that the nerve supplies will not be conducted along the nerve. This would lead to
an increase in the pain threshold. This measurement of the pain sensation of the organ that the nerve supplies, before and after drug administration would give a measure of the local anaesthetic potency of the drug. The tail of rat or mouse is obviously most suitable for this purpose.

The essential oil suspension was made in Tween-80 as to contain 100 mg/ml and varying doses of the essential oil ranging from 100/µg to 2 mg were injected deep into the root of the tail of the mouse so that the oil is in contact with the nerve supplying the tail. The pain sensation of the tail was measured before and after the administration of the drug suspension using the analgesiometer and procedure described before. Control animals received equivalent amounts of the solvent and these experiments were done simultaneously. After drug or solvent injection, the animals were tested at 5, 10, 15 and 30 min.

Effect of the essential oil on the conditioned avoidance response of trained rats

The technique of Cook and Weidley (1956) was followed. The animals were placed in a specially constructed chamber having a grid floor and also a pole in the center. The animals were trained so that they learned to escape by climbing the pole on hearing the sound of a bell in order to avoid an electric shock passed through the grid floor 10 seconds later. This response is termed conditioned avoidance response (CAR). The animals were trained for 2 weeks (2 sessions per day) and only those that responded promptly at the sound of the bell were selected for drug treatment. The trained animals were divided
into 4 groups (10 animals in each group) and were treated with the following: Group I, solvent; Group II, essential oil 140 mg/kg; Group III, 210 mg/kg and; Group IV chlorpromazine hydrochloride, 7.5 mg/kg.

After solvent or drug treatments the animals were again tested at various intervals (15, 30, 60 and 120 min) to see whether there was any disruption in their trained performance. If the CAR was blocked by the drug, the trained animals failed to show their usual response of climbing the pole after the bell sounds, but will nevertheless climb after receiving the electric shock. But if the animal fails to climb the pole even after the electric shock, the drug was considered to block the unconditioned escape response (ER) of the animal.

**Effect of the essential oil of X-alatum on the amphetamine toxicity in aggregated mice.**

It is known that doses of d-amphetamine that do not kill isolated animals cause lethality when the animals are grouped together (aggregated). (Chance, 1946; Burn and Hobbs, 1958)\(^{175,176}\). A number of central depressants are known to protect animals from such death. So in the present study the influence of the essential oil of *X-alatum* on amphetamine toxicity in aggregated mice was studied.

Forty-five animals were used for this study. They were divided into nine equal groups. Six of these groups were treated with the solvent and the rest were treated with the essential oil (400 mg/kg), and were kept in nine cages (28 cm x 19 cm x 12 cm) Fifteen minutes later groups I - III received d-amphetamine sulfate (10 mg/kg), groups IV - VI received d-amphetamine sulfate (20 mg/kg) and groups VII - IX
(essential oil ones) received d-amphetamine sulfate (10 mg/kg). The number of animals found dead in 24 hours was noted in each group.

**Effect of the essential oil on electroconvulsive threshold of rats.**

Fifty rats were used for this experiment. The animals were divided into five equal groups and different groups were treated with 1) solvent, 2) essential oil, (200 mg/kg), e) essential oil (300 mg/kg) 4) essential oil 400 mg/kg, 5) diphenylhydantoin sodium (25 mg/kg). One hour later electroconvulsive shock (ECS) was administered to all the five groups and the number of animals getting extensor spasm and the number of animals dying within 24 hours were noted in each group. It is well known that a maximal electroshock will produce extension of hind limbs (extensor spasm) in all the animals and an anticonvulsant will prevent the animals from getting this seizure (Toman et al., 1946)\textsuperscript{177}. The maximal electroshock will not normally kill any animal, but if the drug potentiates the seizure, it will cause death in a majority of the animals.

For administering electroshock, a convulsimeter was employed and the maximal electroshock (150 m A for 0.2 sec) was administered through ear-clip electrodes, electrode paste being applied in order to increase the conductivity.

**Determination of serotonin, dopamine and norepinephrine in mouse brain**

The brain amines were determined according to the method of Fleming et al., (1965)\textsuperscript{178}. The solvents were prepared as described by them.
Brains, 18 to 22 g from male, Swiss albino mice sacrificed by decapitation, were rapidly removed, rinsed in ice cold isotonic saline, blotted, weighed on a torsion balance and homogenized with the special acetone for 30 seconds in a Vortex high speed homogenizer at 40,000 r.p.m. The acetone tissue ratio was 20:1 V/W. Amine extraction was facilitated by allowing the homogenate to stand for 30 minutes with occasional swirling, and was then centrifuged for 5 minutes at 380 x g and the supernatant fluid decanted into 1.5 x 25 cm screw-capped, round bottom glass centrifuge tubes.

The tubes were placed in a Buchler Rotary "Evapo-Mix" and the extract was evaporated to dryness in vacuo (10 mm Hg) at room temperature. Foaming was avoided by rapid rotational movement before and during the application of vacuum. 8 ml of the 0.01N HCl-equilibrated butanol was then added and the tubes were agitated for 1-2 minutes on a Vortex mixer (Scientific Industries, Inc., N. Y.). To expedite solubilization of the residue, 1 ml of 0.01N HCl and 16 ml heptane were added and the amines were driven into the aqueous phase using agitation on the Vortex mixer for 1 minute.

After centrifuging at 380 x g for 5 minutes to separate the two phases, all but traces of the organic phase was carefully aspirated off. Aliquots of the 1.8 ml aqueous phase resulting from the added HCl and the 0.8 ml dissolved in the butanol phase were analyzed fluorometrically for the amines with the Amino-Bowman spectrophotofluorometer (American Instrument Co., City Md) using an Osram Xenon arc, a 1 P 21 Photomultiplier tube, Slit arrangement No. 5, and fused quartz cuvettes (4.25 ml total volume).
Fluorometric analysis of amines

5-Hydroxytryptamine was determined by its inherent fluorescence in 3N HCl at 300 m$\mu$ excitation and 550 m$\mu$ fluorescence wave lengths (Bogdanski et al., 1956)$^{179}$. A 0.6 ml aliquot of the aqueous phase or 1 ml of the column eluate was used for analysis. An equivalent volume of 6.0 N HCl was added to the aliquots and the samples were read in the spectrophoto fluorometer. A reagent blank was run through the entire procedure and read at 300/550 m$\mu$ was used as the 5-hydroxytryptamine blank. This reading was subtracted from that of the sample to obtain the 5-hydroxytryptamine fluorescence contribution of the unknown or standard.

Dopamine was determined by its oxidation to the highly fluorescent 5, 6-dihydroxyindole. A 0.4 ml aliquot of the aqueous phase was used. This was brought to 1.0 ml and pH 5.2 by adding 1 M sodium acetate-acetic acid buffer at pH 5.2. The aqueous phase was treated as follows: 0.2 ml of 0.02N I$_2$ was added, mixed and after standing for 3 minutes; 0.2 ml of Na$_2$SO$_3$ solution added, mixed and allowed to stand for 3 minutes; 0.4 ml of 5 N HCl-ascorbic acid solution was added, mixed and allowed to stand for 45 minutes. It was read at 330/385 m$\mu$ after subtracting the blank to obtain Dopamine fluorescence contribution.

Norepinephrine was determined by its conversion to the highly fluorescent 3, 5, 6-trihydroxyindole. As in the determination of dopamine, a 0.4 ml aliquot of the aqueous phase was brought to 1.0 ml and pH 5.2. The samples were treated as follows: 0.2 ml of 0.02 N I$_2$ was added, mixed and allowed to stand for 6 minutes; 0.2 ml of 0.025 N Na$_2$S$_2$O$_3$ was added and mixed, 0.4 ml of 5 N NaOH-ascorbic acid solution was added, mixed and allowed to stand for 45 minutes and read at 410/520
μ. The tissue blanks were treated as follows: 0.2 ml of 0.025 N Na₂S₂O₃ was added, mixed and allowed to stand for 6 minutes; 0.2 ml of 0.02 N I₂ was added, and mixed. 0.4 ml ascorbic acid-water reagent was added, mixed and allowed to stand for 45 minutes. The solution was read at 410/520 μ and the blank values were subtracted from the sample values to obtain the norepinephrine-fluorescence contribution. The ascorbic acid-water reagent was used in lieu of alkaline ascorbic acid because the former gives a smaller blank reading.
RESULTS

Effects of the essential oil on the spontaneous motor activity of mice

The results are shown in Fig. 11. A dose of 70 mg/kg did not produce any change in the motor activity of mice. Doses of 140 and 210 mg/kg caused a marked decrease in motor activity, the maximum depression being produced within 30 minutes. At 60 and 120 min the activity of the animals was more or less identical to those of the controls.

Hypnotic effects of the essential oil in rats and mice

The results are shown in Table 9. A dose of 350 mg/kg of the essential oil produced a hypnotic effect in 80 percent of rats and 70 percent of mice. A dose of 400 mg/kg caused hypnosis in all the animals, the mean sleeping time for rats and mice being $14.2 \pm 2.4$ min and $13.8 \pm 1.2$ min respectively. Further increase in the dose failed to prolong the sleeping time further. It may be noted that the duration of sleep was much less than that caused by the ultrashort-acting barbiturate hexobarbitone sodium.

Effects of the major constituents of the essential oil of X-alatum (linalool and methyl cinnamate) in mice

The results are given in Table 10. It may be seen that either linalool or methyl cinnamate administered in the same proportion as is present in the oil fail to produce a hypnotic effect, but, when a
FIG. 11

INFLUENCE OF THE ESSENTIAL OIL ON THE
SPONTANEOUS ACTIVITY OF MICE

ACTIVITY METER COUNTS

CONTROL
ESS. OIL 70 mg/Kg
ESS. OIL 140 mg/Kg
ESS. OIL 210 mg/Kg

0 10 30 60 120
TIME IN MINUTES
Fig. 11  Influence of Essential oil on the spontaneous activity of mice

A dose of 70 mg/kg was not effective in changing the spontaneous activity but marked reduction in activity was produced in doses of 140 and 210 mg/kg. The maximum effect was seen at 10 minutes.
<table>
<thead>
<tr>
<th>Drug and Dose</th>
<th>No animals used</th>
<th>No animal losing righting reflex</th>
<th>Duration of sleep (min±SE)</th>
<th>No animals used</th>
<th>No animals losing righting reflex</th>
<th>Duration of sleep (min±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 mg/kg</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>350 mg/kg</td>
<td>10</td>
<td>8</td>
<td>9.4±3.8</td>
<td>10</td>
<td>7</td>
<td>8.8±3.9</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>10</td>
<td>10</td>
<td>14.2±2.4</td>
<td>10</td>
<td>10</td>
<td>13.8±1.2</td>
</tr>
<tr>
<td>450 mg/kg</td>
<td>10</td>
<td>10</td>
<td>14.9±1.6</td>
<td>10</td>
<td>10</td>
<td>14.1±2.0</td>
</tr>
<tr>
<td>Pentobarbitone sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>10</td>
<td>10</td>
<td>84.4±4.1</td>
<td>10</td>
<td>10</td>
<td>72.1±6.2</td>
</tr>
<tr>
<td>Hexobarbitone sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>10</td>
<td>10</td>
<td>32.6±1.9</td>
<td>10</td>
<td>10</td>
<td>28.0±1.9</td>
</tr>
<tr>
<td>Drug and Dose</td>
<td>No animals</td>
<td>No animals showing hypnotic effects</td>
<td>Duration of sleeping time (mean±S.E.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------------</td>
<td>------------------------------------</td>
<td>-------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Essential oil 400 mg/kg</td>
<td>10</td>
<td>10</td>
<td>13.8±1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl cinnamate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 mg/kg</td>
<td>10</td>
<td>0</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linalool 88 mg/kg</td>
<td>10</td>
<td>0</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl cinnamate + Linalool</td>
<td>10</td>
<td>10</td>
<td>6.6±1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹This amount is equivalent to that present in 400 mg/kg dose of the essential oil.
mixture of these two was given, a hypnotic effect typical of the essential oil was produced.

By increasing the dose, lonalool by itself could cause hypnosis, but methyl cinnamate did not possess this property (Table 10).

Effect of subhypnotic doses of the essential oil on pentobarbitone induced hypnosis in mice

The results are shown in Table 11. It may be seen that the essential oil potentiates the sleeping time due to pentobarbitone sodium. Though the effect produced by 70 mg/kg dose of the oil was not statistically significant, the other two dose levels caused significant enhancement.
## Table 11 - Effects of sub-hypnotic doses of the essential oil on the pentobarbitone-induced hypnosis in mice

<table>
<thead>
<tr>
<th>Drug and Dose</th>
<th>No animals</th>
<th>Sleeping time (Mean±S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% Tween-80 Pentobarb. Sodium 50 mg/kg</td>
<td>10</td>
<td>72.4 ± 2.8</td>
</tr>
<tr>
<td>Essential Oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70 mg/kg + Pentobarb. 50 mg/kg</td>
<td>10</td>
<td>81.1 ± 3.1</td>
</tr>
<tr>
<td>140 mg/kg + Pentobarb. 50 mg/kg</td>
<td>10</td>
<td>114.8 ± 1.4 (P &lt; 0.05)</td>
</tr>
<tr>
<td>210 mg/kg Pentobarb. 20 mg/kg</td>
<td>10</td>
<td>142.4 ± 3.0 (P &lt; 0.01)</td>
</tr>
</tbody>
</table>
Influence of the essential oil on the rectal temperature of mice

The results are shown in Fig. 12. Animals that received 210 mg/kg dose of the essential oil showed a gradual fall in their rectal temperature, the maximum fall of 1.1°C was seen at 30 min, after which the temperature reached normal levels. In a dose of 400 mg/kg, the maximum lowering of temperature was 1.4°C seen at 30 min.

Analgesic effects of the essential oil

The results are given in Table 12. A dose of 70 mg/kg of the essential oil did not produce any analgesic effects in rats, but significant elevation in pain threshold was observed in animals receiving 140 and 210 mg/kg doses of the oil. The effects started within 15 min, reached the maximum at 30 min and subsided within 1 hr. The degree of analgesia produced by 140 mg/kg dose of the essential oil was more or less similar to that of morphine hydrochloride (5 mg/kg) but the latter drug acted for a longer time.

Local anesthetic effect of the essential oil

The results are shown in Table 13. It may be seen that the minimum dose of the oil required to produce a decrease in the pain sensation is 2 mg. A 4 mg dose of the oil caused marked loss of pain sensation, the maximum effect being observed at 10 min. This effect was found to be over when the animals were tested at 30 min.

Effect on the conditioned avoidance response (CAR) of trained rats

A dose of 70 mg/kg of the oil failed to produce any effect in
INFLUENCE OF THE ESSENTIAL OIL ON THE RECTAL TEMPERATURE OF MICE

(FIG. 12)

TEMP. °C

CONTROL
ESS. OIL 210 mg/Kg
ESS. OIL 400 mg/Kg

TIME IN MINUTES
Fig. 12  

**Influence of the Essential oil on the rectal temperature of mice**

In doses of 210 and 400 mg/kg a fall in rectal temperature was produced. The maximum fall was seen at 30 minutes.
<table>
<thead>
<tr>
<th>Drug and Dose</th>
<th>No. animals</th>
<th>Reaction time before drug treatment</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Tween-80)</td>
<td>20</td>
<td></td>
<td>12.2±1.4</td>
<td>11.8±0.9</td>
<td>12.0±1.1</td>
<td>12.1±1.2</td>
</tr>
<tr>
<td>Essential oil 70 mg/kg</td>
<td>10</td>
<td></td>
<td>10.9±1.2</td>
<td>11.1±1.2</td>
<td>12.0±1.6</td>
<td>11.1±1.4</td>
</tr>
<tr>
<td>140 mg/kg</td>
<td>10</td>
<td></td>
<td>13.0±1.1</td>
<td>21.2±1.6</td>
<td>24.2±0.9</td>
<td>13.4±1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P&lt;0.01)</td>
<td></td>
<td></td>
<td>(P&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>210 mg/kg</td>
<td>10</td>
<td></td>
<td>10.6±1.8</td>
<td>28.1±1.8</td>
<td>32.4±2.4</td>
<td>13.9±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P&lt;0.001)</td>
<td></td>
<td></td>
<td>(P&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Morphine HCl 5 mg/kg</td>
<td>10</td>
<td></td>
<td>11.0±1.1</td>
<td>19.4±1.9</td>
<td>23.1±2.6</td>
<td>20.2±1.4</td>
</tr>
</tbody>
</table>
### Table 13 - Influence of the essential oil of X-alatum administered into the root of the tail of the mouse, on the pain sensation of the tail

<table>
<thead>
<tr>
<th>Drug and Dose</th>
<th>No. animals</th>
<th>Reaction time (mean±S.E.) before drug</th>
<th>Reaction time (mean±S.E.) after drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent¹</td>
<td>40</td>
<td>10.9±1.8</td>
<td>11.1±1.1 11.1±1.1 9.8±0.9 11.1±0.9</td>
</tr>
<tr>
<td>Essential oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 g</td>
<td>10</td>
<td>9.4±1.6</td>
<td>10.8±1.2 9.9±0.9 10.1±1.6 10.0±1.2</td>
</tr>
<tr>
<td>1 mg</td>
<td>10</td>
<td>11.2±0.9</td>
<td>10.9±0.9 11.6±0.6 11.8±1.2 10.8±1.1</td>
</tr>
<tr>
<td>2 mg</td>
<td>10</td>
<td>10.8±1.8</td>
<td>14.4±0.9 14.8±1.8 14.0±1.8 11.2±1.9</td>
</tr>
<tr>
<td>4 mg</td>
<td>10</td>
<td>11.0±1.1</td>
<td>18.1±2.0 19.8±1.1 19.8±1.1 11.9±1.1</td>
</tr>
</tbody>
</table>

¹The volume of the solvent injected corresponds with the volume administered with 2 mg dose of the oil.
trained rats, but a dose of 140 mg/kg caused blockade of CAR in 20 percent of the animals. (Table 14). In the highest dose tested (210 mg/kg), the blockade was more marked, 60 percent of the animals showing a blockade of CAR at 15 min. This effect lasted for less than 1 hour. ER was not affected in any of the rats.

Chlorpromazine hydrochloride in a dose of 7.5 mg/kg caused marked effects in trained rats. In this case too the effects started within 15 min but the maximum effect was seen at 30 min (80 percent blockade) and even at 120 min 30 percent of the animals were still under the influence of the drug (Table 14).

Effect of the essential oil on amphetamine toxicity in aggregated mice

The results are given in Table 15. In the solvent-treated groups, 10 mg/kg dose of d-amphetamine sulfate did not cause death in animals, but a dose of 20 mg/kg caused death in 53.3 percent of the animals. Pretreatment of animals with the essential oil potentiated the toxic effect of d-amphetamine (10 mg/kg) in that 73.3 percent of the animals were found dead in these groups (Table 15).

Effect of the essential oil on the electro-convulsive threshold of rats

The results are given in Table 16. All the control animals showed extensor spasm when the maximal electroshock was given. A dose of 300 mg/kg of the oil offered protection to 20 percent of the animals, but 400 mg/kg dose offered marked protection (90 percent) to rats. Diphenylhydantoin sodium (25 mg/kg) a well-known anticonvulsant drug was very effective (100 percent) in this regard.
Table 4 - Effect of the essential oil of X-alatum and Chlorpromazine HCl on the conditioned avoidance response (CAR) of trained rats

<table>
<thead>
<tr>
<th>Drug and Dose</th>
<th>No. animals</th>
<th>CAR</th>
<th>ER</th>
<th>CAR</th>
<th>ER</th>
<th>CAR</th>
<th>ER</th>
<th>CAR</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (solvent)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Essential oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70 mg/kg</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>140 mg/kg</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>210 mg/kg</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chlorpromazine HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5 mg/kg</td>
<td>10</td>
<td>6</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

1CAR - Number of animals which failed to climb the pole after the bell sounded, but did so after receiving the electric shock.

2ER - Number of animals which failed to respond even after receiving the electric shock.
Table 15 - Effect of the essential oil of X-alatum on the amphetamine toxicity in aggregated mice

<table>
<thead>
<tr>
<th>Drug and Dose</th>
<th>Group No.</th>
<th>No. of animals in each group</th>
<th>No. of animals died within 24 hrs.</th>
<th>Percent mortality</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>I</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Solvent + d-amphetamine SO&lt;sub&gt;4&lt;/sub&gt; 10 mg/kg</td>
<td>II</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Solvent + d-amphetamine SO&lt;sub&gt;4&lt;/sub&gt; 20 mg/kg</td>
<td>IV</td>
<td>5</td>
<td>3</td>
<td>60</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>5</td>
<td>3</td>
<td>60</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td>VI</td>
<td>5</td>
<td>2</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Essential oil 400 mg/kg + d-amphetamine SO&lt;sub&gt;4&lt;/sub&gt; 10 mg/kg</td>
<td>VII</td>
<td>5</td>
<td>4</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VIII</td>
<td>5</td>
<td>3</td>
<td>60</td>
<td>73.3</td>
</tr>
<tr>
<td></td>
<td>IX</td>
<td>5</td>
<td>4</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>
Table 16 - Influence of the essential oil of X-alatum on the electro-convulsive threshold of rats

<table>
<thead>
<tr>
<th>Drug and Dose</th>
<th>No. of animals used</th>
<th>No. of animals showing extensor spasm</th>
<th>Percent of animals protected</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (solvent)</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Essential Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>300 mg/kg</td>
<td>6</td>
<td>4</td>
<td>33.3</td>
<td>0</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>10</td>
<td>2</td>
<td>80.0</td>
<td>0</td>
</tr>
<tr>
<td>Diphenylhydantoin Sodium</td>
<td>10</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
</tr>
</tbody>
</table>

Maximal electroshock (150 mA for 0.2 sec) was administered through ear-clip electrodes 60 minutes after drug or solvent treatment.
Brain monoamine level changes

The results are shown in Fig. 13. The changes in the brain DA levels was not statistically significant at any time. NE levels showed a significant (\(P < 0.01\)) elevation at 60 min, but was practically normal at 5.30 and 120 min (\(P > 0.05\) at all the three times). 5-HT level was elevated by 17.5 percent at 5 min which showed a continued size at 30 and 60 min. At 60 min when the effect was maximum the increase in 5-HT level amounted to 40 percent (\(P < 0.01\)) which reached near-normal values at 120 min (\(P > 0.005\)).
EFFECT OF THE ESSENTIAL OIL (400 MG/KG) ON THE MONOAMINE LEVELS OF WHOLE BRAIN OF MICE

(FIG. 13)

AMINE CONC. (% OF SALINE TREATED CONTROLS)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140

TIME IN MINUTES

- DA
- NE
- 5-HT
Fig. 13  Effect of the Essential oil on the monoamine levels of whole brain of mice

In a dose of 400 mg/kg, the essential oil caused an increase of both serotonin (5-HT) and norepinephrine (NE) levels of the whole brain of mice. The maximum increase was seen at 60 minutes. Dopamine (DA) level was not significantly altered.