CHAPTER V

PHARMACOLOGICAL STUDIES OF ESSENTIAL OILS
The essential oils are known to have widely varied pharmacological actions. The pharmacological studies were therefore undertaken with a view to see whether these studies could explain some of the indigenous uses of the rhizomes of *Eucalyptus polyantha*, *Buxus semia*, *Serrana montana*, *Serrana sp.*, *Alpinia galanga*.

The following properties were investigated:

A) Acute Toxicity
B) Effect on Central Nervous System
C) Effect on Cardio Vascular system
D) Effect on Smooth Muscle
E) Effect on Artificially Induced Inflammation
F) Effect on Implantation in Female Rat

Each essential oil was emulsified with 2% aqueous polysorbate-80 (Tween 80) and passed through a hand homogeniser to give a 5% emulsion. Further dilutions were made in physiological solutions or distilled water. Gum acacia was used as emulgent for performing experiments on cardio-vascular system, as polysorbate-80 is known to cause a fall
in blood pressure in anaesthetised dogs due to histamine release.

All the drugs were administered intraperitoneally except where mentioned otherwise and appropriate control experiments were carried out simultaneously wherever necessary. All the experiments were performed in triplicate and at room temperature (25 ± 2°C).

A) Acute toxicity

Graded doses of essential oils were given to groups of albino mice (weight 15 - 35 g). The mortality was recorded after 48 hours.

No mortality was observed with any essential oil up to a dose of 500 mg/kg. The essential oils of C. amada and C. nesbitii did not cause any mortality up to a dose of 500 mg/kg. The mortality was, however, 10% in case of C. nodosa and 20% in case of A. melaleuca and E. nutans with 500 mg/kg doses.

B) Effect on Central Nervous System

1. Gross behaviour of Albino Mice

The albino mice were divided into 4 groups of 5 animals each and kept in separate metallic cages with wire meshed walls. The animals were injected with the essential
oil (75 mg/kg, 150 mg/kg), chlorpromazine hydrochloride (2 mg/kg) and 3% polysorbate-80 (4 ml/kg) respectively. The cages were numbered and the animals were left undisturbed for 30 minutes. The signs of central nervous system depression were observed 30 and 60 minutes after the administration of various drugs and thereafter at hourly intervals for 4 hours (Table X).

The symptoms of central nervous system depression were observed in the groups of mice treated with essential oils (75 mg/kg and 150 mg/kg). No such effect was evident in the animals treated with 3% polysorbate-80 (4 ml/kg).

The essential oils of *E. scabridus* (150 mg/kg) showed marked sedative effect and the spontaneous motor activity was decreased to a great extent. The animals treated with the essential oil moved with unsteady gait and there was marked ptosis. The response to pain and touch stimuli was also reduced but response to sound was not very much affected. The awareness and alertness were markedly affected but the effect on righting reflex was not so marked. The depressant effect lasted for 4 hours with no mortality within 24 hours. The peak action of the essential oil was recorded 30 minutes after administration.

The effects of the essential oils of *E. scabridus*, *E. casina*, *E. raederi* and *A. palmarum* were similar to those
# Table - X

Effect of Essential Oils of Various Rhizomes on Gross Behaviour of Albino Mice

<table>
<thead>
<tr>
<th>Gross Behaviour</th>
<th>A. zelicaria</th>
<th>S. natalensis</th>
<th>S. canina</th>
<th>S. canina</th>
<th>A. zelicaria</th>
<th>Chlorpromazine</th>
<th>Polysorbate 80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor activity</td>
<td>+ 3+</td>
<td>+ 2+</td>
<td>+ 0</td>
<td>+ 0</td>
<td>+ 4+</td>
<td>0</td>
<td>4 mL/kg</td>
</tr>
<tr>
<td>Caut</td>
<td>Normal, unsteady, steady</td>
<td>Normal, unsteady, steady</td>
<td>Normal, unsteady, steady</td>
<td>Normal, unsteady, steady</td>
<td>Normal, unsteady, steady</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sound response</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3+</td>
</tr>
<tr>
<td>Pain response</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2+</td>
</tr>
<tr>
<td>Awareness</td>
<td>+ 3+</td>
<td>+ 2+</td>
<td>+ 2+</td>
<td>+ 2+</td>
<td>+ 2+</td>
<td>+ 4+</td>
<td>0</td>
</tr>
<tr>
<td>Alertness</td>
<td>2+</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
</tr>
<tr>
<td>Sedation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3+</td>
</tr>
<tr>
<td>Touch response</td>
<td>+ 2+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2+</td>
</tr>
<tr>
<td>Convulsions</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>Righting reflex</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>3+</td>
</tr>
<tr>
<td>Mortality</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Eyelid closure

*Key: 0 = No effect, 1+ = slight depressant effect, 2+ = moderate depressant effect, 3+ = severe depressant effect, 4+ = very severe depressant effect.*
of *E. rotundifolia* but of lesser intensity. The essential oils can be arranged in following order of decreasing depressant activity — *E. rotundifolia, C. nodosum, C. sassafras, C. amada, A. calancea*.

2. Rectal Temperature of Albino Rabbits

The albino rabbits were divided into groups of 2 animals each and kept in individual cages. The rectal temperature of the animal was noted by keeping the bulb of clinical thermometer inside the rectum for 60 seconds. The animals of the respective group were injected with the essential oil (75 mg/kg) and 5% polysorbate-80 (4 mL/kg). The third group of animals served as control. The rectal temperature of the animals was again noted 30 and 60 minutes after the administration of the drug and thereafter at hourly intervals for 4 hours.

The essential oil of *A. calancea* caused marked hypothermia. The effect started within 30 minutes of the administration of the essential oil and lasted for about 4 hours, the maximum fall in rectal temperature was $4.2^\circ\text{C}$ with 75 mg/kg dose. The animal receiving 5% polysorbate-80 did not show any lowering of the rectal temperature. The essential oils of *E. rotondifolia, C. sassafras, C. amada* and *C. nodosum* also produced hypothermia in test animals, the effect was however less marked (Table - XI).
### Table - XI

**Effect of Essential Oils of Various Rhizomes on Rectal Temperature of Albino Rabbit**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Temperature Before drug administration</th>
<th>Temperature After drug administration 30 min</th>
<th>Temperature After drug administration 1 hr</th>
<th>Temperature After drug administration 2 hr</th>
<th>Temperature After drug administration 3 hr</th>
<th>Temperature After drug administration 4 hr</th>
<th>Maximum fall in rectal temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil of <em>L. umbumia</em> (75 mg/kg)</td>
<td>102.6</td>
<td>101.2</td>
<td>100.4</td>
<td>100.6</td>
<td>101.2</td>
<td>102.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Essential oil of <em>G. minima</em> (75 mg/kg)</td>
<td>104.0</td>
<td>102.2</td>
<td>103.0</td>
<td>103.6</td>
<td>103.6</td>
<td>103.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Essential oil of <em>G. scabiana</em> (75 mg/kg)</td>
<td>103.0</td>
<td>102.0</td>
<td>101.6</td>
<td>101.4</td>
<td>101.6</td>
<td>101.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Essential oil of <em>G. salvia</em> (75 mg/kg)</td>
<td>103.0</td>
<td>101.6</td>
<td>101.4</td>
<td>101.6</td>
<td>102.4</td>
<td>102.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Essential oil of <em>A. salina</em> (75 mg/kg)</td>
<td>103.6</td>
<td>100.4</td>
<td>99.4</td>
<td>100.4</td>
<td>100.4</td>
<td>101.6</td>
<td>4.2</td>
</tr>
<tr>
<td>3% polysorbate-80 Control 4 mL/kg</td>
<td>104.0</td>
<td>105.6</td>
<td>104.0</td>
<td>103.6</td>
<td>104.0</td>
<td>103.6</td>
<td>-</td>
</tr>
</tbody>
</table>
5. Pentobarbitone-induced Hypnosis in Albino Mice

The albino mice (20 - 25 g) were divided into 3 groups of 10 animals each. The first and second group of animals were administered 75 mg/kg and 150 mg/kg dose of essential oil respectively. The third group received only 0.5% polysorbate-80 (4 ml/kg). Fifteen minutes latter, animals of all the groups were given 25 mg/kg dose of pentobarbitone sodium. The animals were gently put on their backs when they felt asleep. The time of loss and return of righting reflexes was noted and the recovered animals removed to avoid disturbance to other animals.

The essential oil of *K. rotunda* (75 mg/kg and 150 mg/kg) potentiated the hypnosis caused by pentobarbitone sodium increasing the mean sleeping time (28.6% and 63.1%).

The effect of the essential oils of *K. sativa* and *G. amada* was close to that of *K. rotunda* but it was even lesser in case of *G. sesquipedalis* and *G. coccina* (Table - XII).

4. Rotated Performance of Albino Rats

The albino rats were trained to remain for a minimum period of 2 minutes on a cylinder (diameter 15 cm) revolving horizontally at the rate of 4 revolutions per minute. The animals were trained initially before studying the effect of essential oil and divided into 4 groups of 10 each. First
Table XII
Effect of Essential Oils of Various Rhizomes on Pentobarbitone-induced Hypnosis in Albino Mice

<table>
<thead>
<tr>
<th>Dose</th>
<th>Average Sleeping time in minutes</th>
<th>Probability Increase factor</th>
<th>Probability factor of dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>90.2 ± 5.0</td>
<td>20.51</td>
<td></td>
</tr>
</tbody>
</table>

Essential oil of *E. nutansia*
75 mg/kg
+ Pentobarbitone sodium
25 mg/kg

Essential oil of *E. nutansia*
150 mg/kg
+ Pentobarbitone sodium
25 mg/kg

Essential oil of *C. eddoxana*
75 mg/kg
+ Pentobarbitone sodium
25 mg/kg

Essential oil of *C. eddoxana*
150 mg/kg
+ Pentobarbitone sodium
25 mg/kg

Essential oil of *C. eddoxana*
75 mg/kg
+ Pentobarbitone sodium
25 mg/kg

(Contd.)
<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Essential oil of C. ammos</strong></td>
<td>150 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>$103.4 \pm 5.4$</td>
<td>$54.54$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pentobarbitone sodium</strong></td>
<td>25 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$84.62$</td>
<td>$20.69$</td>
<td></td>
</tr>
<tr>
<td><strong>Essential oil of C. ammos</strong></td>
<td>75 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>$102.4 \pm 5.9$</td>
<td>$46.08$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pentobarbitone sodium</strong></td>
<td>25 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Essential oil of A. melaleuca</strong></td>
<td>75 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>$90.6 \pm 4.2$</td>
<td>$20.32$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pentobarbitone sodium</strong></td>
<td>25 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Essential oil of A. melaleuca</strong></td>
<td>150 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>$99.6 \pm 5.6$</td>
<td>$56.53$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pentobarbitone sodium</strong></td>
<td>25 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pentobarbitone sodium</strong></td>
<td>25 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$7%$ polyaerbeate-80</td>
<td>4 mL/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$70.1 \pm 5.2$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and second group of rats were injected with essential oil (75 mg/kg and 150 mg/kg). Third group was given chlorpromazine hydrochloride (5 mg/kg) whereas the rats of control group were given 3% polysorbate-80. The animals were tested for their rotarod performance 30 and 60 minutes after the administration of various drugs and thereafter at hourly intervals for 4 hours. The number of animals falling down during 2 minutes test period was noted.

None of the essential oils markedly affected the rotarod performance of the animals. The essential oil of L. rutlandica, L. rechingeri and L. salmeron, however, caused 40 to 50% animals to fall with the dose of 75 mg/kg and 150 mg/kg respectively. The essential oil of L. oenodia and L. anides had practically no effect on rotarod performance.

5. Pain Threshold of Albino Rats

The experiment was performed by a hot nichrome wire analgesiometer which was provided with an arrangement to circulate cold water to avoid heating the area surrounding the wire. Each albino rat was placed in rat holder and the tail (about 20 mm of the tip) was kept over the wire without actually touching it and the normal reaction time in which the animal withdrew its tail with a jerk was noted.

The rats were then divided into 5 groups of 10 each. The animals of first and second group were administered with
75 mg/kg and 150 mg/kg dose of essential oil, the third group morphine hydrochloride and the fourth morphine hydrochloride and the essential oil and the fifth 3% polysorbate-80. The reaction time of animals was noted 30 and 60 minutes after drug administration and thereafter at hourly intervals for 4 hours.

The essential oils did not exhibit any significant analgesic effect and also did not potentiated the analgesic action of morphine hydrochloride.

c) Effect on Cardiovascular System

Blood Pressure and Respiration of Anaesthetised Dog

The dog was anaesthetised by injecting sodium barbital (30 mg/kg). The femoral vein was exposed, cannulated and connected with a rubber tubing to a burette having normal saline. A midline incision was made on the neck above the trachea, the trachea exposed and one end of tracheal cannula inserted. The other end of the cannula was connected to a Hare’s tambour by rubber tubing for recording respiratory changes. The left common carotid artery was then exposed, clipped at a lower end, and arterial cannula inserted. The other end of the arterial cannula was connected to the mercury column.

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* Longworth dogs weighing between 8 - 10 kg were used for the pharmacological studies.
manometer with a rubber tubing filled with a 10% solution of sodium citrate. The clip on the carotid artery was released and blood pressure was recorded on slowly moving kymograph with a pointer floating on the mercury column. The essential oils (15 mg/kg and 30 mg/kg) was then administered intravenously through the cannulated femoral vein and the effects were recorded (Fig. 26). The control experiments were performed with gum acacia.

All the essential oils produced a fall in the blood pressure in the anaesthetized dog, the effect being at its peak after about 2 to 5 minutes. A decrease in pulse rate was also observed after the administration of essential oils. The respiration was reflexly stimulated for a short duration as evidenced by the increase in the respiration rate and amplitude.

D) Effects on Smooth Muscle

1. Isolated Uterus of Rat

The female nonpregnant albino rat was stunned and bled to death by cutting carotids. The viscera was exposed, the intestinal loops were shifted to one side and one horn of the uterus was removed and suspended in a 10 ml aerated organ bath having modified Ringer's solution which was maintained at 31°C. The contractions produced by oxytocin (0.01 units) were recorded on a kymograph. The essential oil (1 mg) was added to oxytocin treated tissue and the effects recorded again (Fig. 27).
Fig. 26  :  Effect of essential oils of various rhizomes (15 mg/kg and 30 mg/kg) on blood pressure and respiration of anaesthetised dog.

Fig. 27  :  Effect of essential oils of various rhizomes (1 mg) on oxytocin-induced contraction of isolated uterus of rat.
None of the essential oils (1 mg) produced any change in oxytocin response when allowed to remain in contact with the isolated rat uterus for one minute.

2. **On Histamine-induced Spasm of Lung Strip of Dog**

Lulich (1976)\textsuperscript{10} first described an *in vitro* technique using isolated lung parenchyma of cats to measure the effect of drugs on terminal airways. Recently Peter, *et al.*\textsuperscript{11} have described the evaluation of direct effects of bronchodilator and bronchoconstrictors, particularly antiasthmatic drugs using lung strip of domestic animals such as dog, cat and horses.

The method described by Lulich was used for evaluating effect of essential oils on histamine induced spasm on lung strip of dog. A mongrel dog was anaesthetized and dissected to remove lung. The lung was placed in a dissecting tray and the diaphragmatic lobe was identified. Three parallel incisions about 3 mm apart were made to get two parallel strips of 4 - 5 cm, the central 3 cm portion was identified and incised. A pair of curved surgical scissors was then used to remove the two strips at a depth of approximately 3 mm, thus twin strips were prepared for mounting.

The strips were incised parallel to the main bronchus and were chosen from a site which had a normal appearance and
contained no visible bronchi or major blood vessels. Threads were attached to both ends of the strip which was mounted in 20 ml organ bath containing Kreb's solution at 37°C mixed with 95% and 5% oxygen and carbondioxide respectively. A resting isometric tension of 2 - 3 g was applied to all the tissue, which were then allowed to equilibrate for at least 1 hour, the bath fluid being changed at 20 minute intervals. Histamine was added (2 μg) to the bath and after the strip had responded of essential oil (2 mg) was added at different intervals to observe the cumulative effect (Fig. 23).

None of the essential oils neither blocked nor combated the contractile effect of histamine.

3. Effect on Isolated Tracheal Chain of Dog\textsuperscript{12,13,14}

Traditional methods for assessing the bronchial activity of drugs \textit{in vitro} have mainly used tracheal smooth muscle. The dog was anaesthetised by injecting seconal sodium (50 mg/kg). A mid line incision was made on the neck above the trachea, the trachea exposed and 4 - 5 cm piece of the trachea was quickly removed in cold saline. Strips (4 x 0.5 cm) were cut from the tracheal piece and threads were attached to both the ends of the strips. The strip was mounted in 20 ml oxygenated organ bath containing Kreb's solution at 37°C and contractions recorded. The bath liquid was changed at every 20 minutes intervals, until
Fig. 28: Effect of essential oils of various Rhizomes (2 mg) on histamine-induced spasm of lung strip of cow.

Fig. 29: Effect of essential oils of various Rhizomes (1 mg) on isolated tracheal chain of cow.
regular contractions were obtained. Histamine (1 µg) was then added to the bath and the spasms recorded. When the tissue response was at its peak, the essential oil (1 mg) was added and its effect recorded (Fig. 39).

The essential oils tested did not significantly block the spasm produced by histamine, the intensity of spasms was, however, controlled to half when essential oils of C. amada and K. rotunda were administered. The other essential oils did not show this effect.

E) Effect on artificially induced Inflammation

The method recommended by Buttke, et al. was followed. A modified 2 ml microburette was connected to a 5 ml glass syringe through a glass 'U' tube. The lower end of the burette and the tube was filled with mercury. The upper portion of the microburette was filled with water. The burette was graduated with two marks 'A' and 'B' respectively 1.5 and 5 cm below the upper end.

The albino rats were divided into 2 groups of 5 animals each and injected with essential oils (150 mg/kg) and 3% polysorbate-80 (4 ml/kg) respectively. The paw volume of the animals was measured and carrageenan was injected subcutaneously in the right hind paw of all the rats 1 hour after administration of essential oil. Three hours after the injection of carrageenan, paw volume of the animal was measured again.
The paw volume was measured in the manner described below. The level of water was first adjusted to mark 'A' with the help of the syringe and the reading of water-mercury interface was observed on the burette scale. The foot of the animal was then introduced into the burette until the tip of the foot coincided with mark 'B', thus causing the water level to rise. The piston of the syringe was then withdrawn to bring the water-level back to mark 'A', the reading of the water-mercury interface being again observed. The difference between the two observations of water-mercury interface represented the volume of the animal's foot (Table - XIII).

The essential oils exhibited mild significant activity against carrageenan induced inflammation in rats.

F) **Effect on Fertility**

**Effects on Implantation in Female Rats**

Female rats (Charles-Foster Strain) in estrus phase were caged together with male rats in the ratio of 1:1 in air conditioned rooms with 14:10 hours dark:light schedule. Vaginal smears of the female rats were prepared the following morning and observed microscopically. Female rats showing thick clumps of spermatozoa in vaginal smears were removed and considered to be pregnant from that day. Pregnant female rats were divided in groups of 5 each and placed in separate cages. They were then treated with aqueous emulsion of the
Table - XIII
Effect of Essential Oils of Various Rhizomes on Carrageenan induced Inflammation in Albino Rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. of animals employed</th>
<th>Inflammation %</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil of <em>K. rotunda</em> 150 mg/kg</td>
<td>5</td>
<td>53</td>
<td>42</td>
</tr>
<tr>
<td>Essential oil of <em>C. amada</em> 150 mg/kg</td>
<td>5</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>Essential oil of <em>C. zedoaria</em> 150 mg/kg</td>
<td>5</td>
<td>63</td>
<td>32</td>
</tr>
<tr>
<td>Essential oil of <em>C. casia</em> 150 mg/kg</td>
<td>5</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Essential oil of <em>A. galanga</em> 150 mg/kg</td>
<td>5</td>
<td>53</td>
<td>42</td>
</tr>
<tr>
<td>Polysorbate-80 3% 4 ml/kg Control</td>
<td>5</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>
essential oils (250 mg/kg) orally from first day to seventh day of pregnancy.

The animals were laprotomized on 10th day and the number of implants in the two horns of the uterus were counted (Table - XIII). The rats were sutured and allowed to complete the term for maturation of wombs. The litter size was then checked and the pups were observed for one month for any gross teratogenic effect. A control group of the pregnant rats treated with only the gum acacia suspension was also maintained (Table - XIV).

All the essential oils except that of G. caesia inhibited implantation in 20% animals in a dose of 250 mg/kg. The essential oil of G. caesia (250 mg/kg) inhibited implantation in 60% of the animals under test. Increased dose of essential oil of G. caesia (500 mg/kg) surprisingly checked implantation in 20% animals only. No teratogenic effect was observed in the offsprings during one month.
### Table - XIV

**Anti-fertility Screening of Essential Oils of Various Rhizomes**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Dose</th>
<th>Route</th>
<th>No. of pregnant Rats used</th>
<th>No. of rats with implants</th>
<th>Anti-implantation activity %</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Aqueous Cum acacia Suspension 250 mg/kg</td>
<td>P/o</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>No activity</td>
</tr>
<tr>
<td>2</td>
<td>Essential oil of <em>A. galanga</em> 250 mg/kg</td>
<td>P/o</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>Not significant</td>
</tr>
<tr>
<td>3</td>
<td>Essential oil of <em>C. zedoaria</em> 250 mg/kg</td>
<td>P/o</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>- do -</td>
</tr>
<tr>
<td>4</td>
<td>Essential oil of <em>C. amada</em> 250 mg/kg</td>
<td>P/o</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>- do -</td>
</tr>
<tr>
<td>5</td>
<td>Essential oil of <em>K. rotunda</em> 250 mg/kg</td>
<td>P/o</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>- do -</td>
</tr>
<tr>
<td>6</td>
<td>Essential oil of <em>C. caesia</em> 250 mg/kg</td>
<td>P/o</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>Significant</td>
</tr>
<tr>
<td>7</td>
<td>Essential oil of <em>C. caesia</em> 500 mg/kg</td>
<td>P/o</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

In all cases no evidence of teratogenic effect was observed.
Discussion

The pharmacological investigations of essential oils of *K. rotunda*, *K. annua*, *K. eucalyptus*, *K. nobilis* and *A. salama* were carried out with a view to investigate the reported claims regarding the use of the plants in indigenous medicine. The studies were, therefore, restricted only to those pharmacological actions which may verify the use of the plants.

Acute toxicity studies on albino mice indicated that none of the essential oils produced any mortality up to a dose of 300 mg/kg. This suggests the essential oils have a wide margin of safety.

Various experiments were performed to study the effects of essential oils on central nervous system. The essential oils produced a syndrome of central nervous system depression in dose of 75 mg/kg and 150 mg/kg. The motor activity was inhibited, the animals moved with unsteady gait and there was marked ptosis. The sound, touch, pain responses and righting reflex were also slightly affected. The general awareness and alertness of the animals was affected markedly. Depression, however, was considerably less than that of chlorpromazine. The essential oil of *K. rotunda* produced maximum depression. The essential oils of *K. annua*, *K. eucalyptus*, *K. nobilis* and *A. salama* were similar in actions to those of *K. rotunda*, differing only in the degree of depression. The essential oil of *A. salama* was least effective.
The essential oils produced hypothermia in rabbits. The essential oil of *A. malaccense* was most effective and produced a fall of 4.2°C in the case of 75 mg/kg. The essential oil of *E. rotundifolia*, *E. arborea*, *E. cascorn* and *E. reticulata* also produced hypothermia although to a lesser degree (2.2°C, 1.3°C, 1.4°C, 1.4°C respectively).

All the essential oils potentiated pentobarbitone induced hypnosis. The essential oil of *E. rotundifolia* was most effective and enhanced the mean sleeping time by 28.6 and 65.6% in the case of 75 mg/kg and 150 mg/kg respectively. The hypnosis potentiating effect of essential oils of *A. malaccense* and *E. arborea* was close to that of *E. rotundifolia*.

None of the essential oils produced any marked effect on rotated performance of the animals suggesting thereby that the learned behaviour of the animals was not changed significantly. The essential oils did not increase the pain threshold of albino rats. The morphine induced analgesia was also not potentiated. This rules out the possibility of the presence of analgesic activity in the essential oils.

Although the essential oils did not produce general depression, they enhanced the hypnotic actions of other drugs. The reduction in body temperature of animals is also suggestive of central nervous system depressant property.
The essential oils (30 mg/kg) produced a fall in blood pressure of anaesthetised dogs. The fall was found to be dose dependent. The respiration, however, was reflexly increased for a short duration. The recovery was prompt and complete.

None of the essential oils produced any change in oxytocin response when allowed to remain in contact with isolated rat uterus. The essential oils neither blocked nor combated the contractile effect of histamine on isolated lung strip of dog. The essential oils also did not significantly block histamine induced spasm in isolated tracheal chain of dogs. The essential oils of *E. canidea* and *A. cataphana* were however, able to control the intensity of histamine spasm to half.

The essential oils were tested on carrageenan induced inflammation in albino rats. The inflammation was reduced in all cases, but the inhibition was mild significant (32 to 42%).

The effect of essential oil on implantation in female rats was studied. All the essential oil except that of *E. canidea* inhibited implantation in 20% animals in a dose of 250 mg/kg. The essential oil of *E. canidea* (250 mg/kg) inhibited implantation in 60% animals under test. The increased dose of this essential oil (500 mg/kg) surprisingly checked the implantation in 20% animals only. The observations need to be further investigated.
The pharmacological studies as carried out have clearly demonstrated the presence of depressor properties on C.N.S. The significant hypotensive action and lack of acute toxicity suggest that the essential oils can be useful in thrombosis and febrifuge and also for producing sedation in patients. The essential oils also have hypotensive action but it is difficult to suggest their use in hypertensives unless some clinical evaluation is made.

The anti-inflammatory effect observed with different essential oils is also of value and substantiates the use of these drugs in Ayurvedic practice as anti-inflammatory agent. The observations further indicate that the presence of the anti-inflammatory property is due to the essential oil content of these drugs.
References


