CHAPTER - 4
CHAPTER - 4

PHARMACOLOGICAL STUDIES

4.0 CNS activity

4.1 Introduction

Stress is a physiological phenomenon, which has not been defined clearly. However an attempt has been made to define it as “the sum of all non specific changes caused by function or damage”. It is fundamentally a physiological response whose primary object is to maintain life and re-establish normal life.

The word stress has many connotations: However scientifically speaking any stimulus whether physical, chemical, biological or emotional nature can cause stress. Small degree of stress is needed for normal physiological functions of the organism, but when it is severe and persists it leads to various problems such as hypertension, diabetes, myocardial infarction, bronchial asthma, arthritis, malignancies, ulcer etc. Berkhman and Dardymov (1969) have shown that certain plant extracts possess significant antistress activity in animals and man

Adaptability is the term used for the potential of the individual to adjust mentally and physically to the environment. This potential is influenced by many factors. Adaptogenic herbal drugs strengthen our adaptability to the environmental exposure.

The reactivity of the living cells declines with age and adaptogens appear to increase the reactivity. Protein synthesis, immunity and metabolic regulatory mechanism, which decline due to disease or ageing, appear to be stimulated by these drugs. The three stages of adaptation syndrome are alarm, resistance and exhaustion, through which the body adopts it against the stress. The drugs which help in this
adaptive process and allow the individual to cope with successfully are called as “anti-stress drugs”.

Antipsychotic drugs improve the mood and behavior of psychotic patients without excess sedation and without causing drug dependence. They relieve signs and symptoms of schizophrenia. Positive symptoms like thought-disorder, agitation, delusion; hallucination tend to respond better than negative symptoms like blunted affect, diminished capacity for pleasure, lack of drive and poverty of speech. The forced swimming induced immobility method described by Porsolt et al., (1978) was followed here.

Pain is a subjective experience hard to define exactly even though everyone knows what is meant by it. It is a direct response to an untoward event associated with tissue damage such as injury, inflammation or malignancy but severe pain may arise independently of any obvious predisposing cause like trigeminal neuralgia, or it can occur as a consequence of brain or neural injury. Pain therefore can be explained in terms of disordered neuronal function, comparable to schizophrenia or epilepsy. It is believed that current analgesia-inducing drugs such as opiates and non-steroidal anti-inflammatory drugs (NSAIDs) are not useful in all cases because of their side effects and low potency. As a result, a search for alternatives seems necessary and beneficial. The studies of plant medicine have been traditionally used, as painkiller is still a fruitful and logical way of search for new analgesic drugs.

The effects of ethanolic extract of some of the Indian medicinal plants have been tested for CNS activity. They are Sida acuta (whole plant), Stylosanthes fruticosa (whole plant), Toona ciliata (heart wood), Bougainvilla spectabilis (leaves), Ficus glomerata (bark, leaves) and Polyalthia longifolia (leaves).

These plants have been reported to possess various pharmacological activities such as antiviral, acetylcholine-like activity, antihepatotoxic, antipyretic etc. Ficus
glomerata used in diabetes, anti-inflammatory and piles. T.ciliata bark has been used in chronic dysentery and as external application for ulcers. B.spectabilis has been claimed to be useful in the frequent of diabetes mellitus, antimalarial and anticancer and also possess immunomodulating properties 8-13.

In the present study with rodents concentrated ethanolic extracts were evaluated for anti-stress activity by “forced swimming test” (FST) and locomotor activity by actophotometer and for analgesic activity by hot plate method and tail immersion method.

4.2 Acute toxicity studies

Acute toxicity studies were carried out using acute toxic class method as per OECD guidelines 42514. This method was adopted in 1996 was extensively validated both nationally and internationally. It is a step wise procedure involving use of three animals, and an average of 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This method is based on biometric evaluation with fixed doses (5,50,300,2000mg/kg, p.o) adequately separated to label substance to be ranked for classification purpose. (Appendix D)

Acute toxicity for various plant extracts was carried out using groups of three Swiss albino mice by administering a dose 2000mg/kg, in 1% CMC p.o. and observed for mortality and behavioral changes during 48 h. The results showed no clinical signs and mortality of the animal therefore an LD₅₀ >5000mg/kg body weight may be assumed. It comes under class x – unclassified.

Hence the dose (100mg/kg, 300mg/kg and 500mg/kg) selected for the following studies.
Table 4.0

GROSS BEHAVIOURAL STUDY (OECD Guideline 423)

<table>
<thead>
<tr>
<th>Plant Extract*</th>
<th>Weight of Animals (gms)</th>
<th>Dose (g/kg)</th>
<th>Central Nervous System</th>
<th>Autonomic Nervous System</th>
<th>Motor Activity</th>
<th>Salivation</th>
<th>Skin Colour</th>
<th>General</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Test</td>
<td>After Test</td>
<td>Tremors</td>
<td>Chock</td>
<td>Convulsion</td>
<td>Sedation</td>
<td>Hypnotis</td>
<td>Catatonia</td>
<td>Irritability</td>
</tr>
<tr>
<td>P1</td>
<td>24</td>
<td>24</td>
<td>2000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>P2</td>
<td>22</td>
<td>22</td>
<td>2000</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>P3</td>
<td>20</td>
<td>20</td>
<td>2000</td>
<td>-</td>
<td>+</td>
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<td>-</td>
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<tr>
<td>P4</td>
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<td>21</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>P5</td>
<td>23</td>
<td>23</td>
<td>2000</td>
<td>-</td>
<td>+</td>
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<tr>
<td>P6</td>
<td>22</td>
<td>22</td>
<td>2000</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P7</td>
<td>21</td>
<td>21</td>
<td>2000</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* P1 = S.acuta  
  P2 = S.fruticosa  
  P3 = T.ciliata  
  P4 = B.spectabilis  
  P5 = F.gomerata (bark)  
  P6 = P.longifolia  
  P7 = F.gomerata (leaves)
4.3 Materials and Methods

Animals

Wistar albino rats of either sex / Swiss albino mice were obtained from Tamilnadu Veterinary College and Research Institute, Chennai. The animals were maintained in colony cages at 25 ± 2°C, relative humidity 50-55% maintained under 12 h light and dark cycle (06 to 10 h light 18 to 06 h dark). The animals were fed with Standard animal feed (Hindustan Lever Ltd.) and water was applied ad libitum. All the animals were acclimatized to the laboratory conditions prior to experimentation. The extracts were suspended in 1% Carboxymethylcellulose and administered to the animal’s p.o.

Drugs and Chemicals

Caffeine 50mg/kg - Yarrow Chem Product enterprises, Domvili.

Diazepam 10mg/kg - Ranbaxy Laboratories Ltd, New Delhi.

Chlorpromazine 25mg/kg - Sun pharmamaceutical industries Ltd., Mumbai.

Pentazocine – (fortwin) inj 30mg/ml Ranbaxy Laboratory Ltd., New Delhi, India.

Ranitidine 50mg/kg - Glaxo Pharma, Mumbai, Maharashtra.

Sucralfate 1000mg/kg - (Sucrafil.T) 10mg, Fourrts (India) Laboratories Pvt., Ltd., Chennai.

Omeprazole 20mg/kg- (Omez.T) 30mg, Dr.Reddys Laboratories Ltd, Hyderabad-500066.

Topfers reagent, Phenolphthalein, I.P, Sodium hydroxide, I.P.,
Carboxymethylcellulose, Tween 80 - S.D Fine chemicals.
Test plant extracts

* Sida acuta* (whole plant)

* Stylosanthes fruticosa* (whole plant)

* Toona ciliata* (heart wood)

* Bougainvilla spectabilis* (leaves)

* Ficus glomerata* (bark)

* Polyalthia longifolia* (leaves)

* Ficus glomerata* (leaves)

4.4 Antidepressant activity

**Forced swimming induced immobility in rats**

One hundred and forty four rats deprived of food (water *ad libitum*) for 24 h were randomly grouped into twenty-four groups, each consisting of 6 animals.

Group 1: received 1 ml/kg, p.o 1% CMC as vehicle control

Group 2: received diazepam 10 mg/kg, p.o as standard control (depressant)

Group 3: received caffeine 50mg/kg, p.o as standard control (stimulant)

Group 4-6: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *S.acuta*

Group 7-9: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *S.fruticosa*
Group 10-12: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *T.ciliata*

Group 13-15: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *B.spectabilis*

Group 16-18: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *F.glomerata* (bark)

Group 19-21: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *P.longifolia*

Group 22-24: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *F.glomerata* (leaves)

Forced swimming induced immobility model was used to produce fatigue syndrome. In this rats were forced to swim in a glass cylinder (height 45cm diameter, 25 cm) containing water to a height of 35cm at 35º-37ºC for 6min. daily for 7 days. The FST is a behavioural test widely used to screen new antidepressant drugs in rats and mice\textsuperscript{15, 16}. On the test day, 60 min following treatment rats from each group were dropped individually into the water in glass cylinder. During this period of observation the rat exhibits a distinct type of active behaviors immobility as described by Detke et al. and Bopaiah et al.\textsuperscript{17, 18}. After 2 min, the scorer who is unaware of the treatment recorded the immobility-behavior during the following 4-min. using a stopwatch. Immobility of a rat was judged, when it remained floating in the water without struggling and was making only those movements necessary to keep its head above water. Immobility time is noted, the data expressed in seconds and the values as percentage change in activity considering the vehicle control as 100% as shown in the Table 4.1 and Fig.4.1
<table>
<thead>
<tr>
<th>Treatment</th>
<th>At 100 mg/kg</th>
<th>At 300 mg/kg</th>
<th>At 500 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean immobility time (Sec.) ± SEM</td>
<td>% change in activity</td>
<td>Mean immobility time (Sec.) ± SEM</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>70.83 ± 4.362</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diazepam 10 mg/kg</td>
<td>192.33 ± 8.313**</td>
<td>63.17 ↑</td>
<td>-</td>
</tr>
<tr>
<td>Caffeine 50 mg/kg</td>
<td>14.667 ± 1.626**</td>
<td>79.3 ↓</td>
<td>-</td>
</tr>
<tr>
<td>S.acuta</td>
<td>42.833 ± 2.522**</td>
<td>39.5 ↓</td>
<td>80.5 ± 5.012</td>
</tr>
<tr>
<td>S.fruticosa</td>
<td>78.667 ± 4.216**</td>
<td>9.9 ↑</td>
<td>120.50 ± 6.423**</td>
</tr>
<tr>
<td>T.ciliata</td>
<td>165.833 ± 10.078**</td>
<td>57.3 ↑</td>
<td>126.67 ± 6.412**</td>
</tr>
<tr>
<td>B.spectabilis</td>
<td>176.50 ± 7.588**</td>
<td>59.8 ↑</td>
<td>161.00 ± 7.937**</td>
</tr>
<tr>
<td>F.glomerata (bark)</td>
<td>160.83 ± 5.624**</td>
<td>56.0 ↑</td>
<td>141.17 ± 5.089**</td>
</tr>
<tr>
<td>P.longifolia</td>
<td>145.50 ± 4.978**</td>
<td>51.3 ↑</td>
<td>160.67 ± 8.617**</td>
</tr>
<tr>
<td>F.glomerata (leaves)</td>
<td>165.00 ± 8.540**</td>
<td>57.0 ↑</td>
<td>154.50 ± 9.573**</td>
</tr>
</tbody>
</table>

- ↑ increase in immobility, ↓ decrease in immobility
Each value is the mean ± SEM of 6 determinations
**P < 0.01 dunnet test as compared to control value.
Fig. 4.1

Effects of various plant extracts on mean immobility time as tested by forced swimming at room temperature

Mean immobility time in seconds

- Vehicle control
- Diazepam 10mg
- Caffeine 50mg
- S. acuta
- S. fruticosa
- T. ciliata
- B. spectabilis
- F. glomerata (bark)
- P. longifolia
- F. glomerata (leaves)

Legend:
- 100mg/kg
- 300mg/kg
- 500mg/kg
4.5 Locomotor activity

One hundred and thirty eight rats deprived of food (water *ad libitum*) for 24 h were randomly grouped into twenty-three groups, each consisting of 6 animals.

Group 1: received chlorpromazine 25 mg/kg, p.o as standard control (depressant)

Group 2: received Caffeine 50mg/kg, p.o as standard control (stimulant)

Group 3-5: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *S.acuta*

Group 6-8: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *S.fruticosa*

Group 9-11: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *T.ciliata*

Group 12-14: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *B.spectabilis*

Group 15-17: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *F.glomerata* (bark)

Group 18-20: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *P.longifolia*

Group 21-23: received 100mg/kg, 300mg/kg, 500mg/kg, p.o of ethanolic extract of *F.glomerata* (leaves)

Most of the CNS acting drugs influence the locomotor activities in man and animals. The CNS depressant drug such as barbiturates and alcohol reduce the motor activity...
activity while the stimulants such as caffeine and amphetamine increase the activity. Wistar albino rats of either sex selected by random sampling technique were used for this test. The locomotor activity (horizontal activity) was measured using an actophotometer, which operates on photoelectric cell, is connected in circuit with a counter. When the animal cuts off the beam of light falling on the photocell the interruption of a beam generates an electric impulse and count was recorded\textsuperscript{19-21}.

Rats were individually placed in the activity cage for 10 min. Basal activity score of each animal was noted. After 60 min, every animal was retested for activity scores for another 10 min. The difference between the pre- and post-treatment activity was recorded and the data were expressed as change in activity. Table 4.2 and Fig. 4.2
Table 4.2
Effect of various plant extracts on locomotor activity score in rats as tested in actophotometer
Mean locomotor activity score

<table>
<thead>
<tr>
<th>Treatment</th>
<th>At 100 mg/kg</th>
<th>% Change in activity</th>
<th>At 300 mg/kg</th>
<th>% Change in activity</th>
<th>At 500 mg/kg</th>
<th>% Change in activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Locomotor Score ± SEM</td>
<td></td>
<td>Mean Locomotor Score ± SEM</td>
<td></td>
<td>Mean Locomotor Score ± SEM</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine 25 mg/kg</td>
<td>265.166 ± 9.38***</td>
<td>76.34 ↓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Caffeine 50 mg/kg</td>
<td>205.833 ± 13.245***</td>
<td>41.69 ↑</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. acuta</td>
<td>97.166 ± 8.348***</td>
<td>26.74 ↑</td>
<td>61.166 ± 4.556***</td>
<td>21.14 ↑</td>
<td>73.50 ± 7.371**</td>
<td>23.35 ↑</td>
</tr>
<tr>
<td>S. fruticosa</td>
<td>84.0 ± 7.84***</td>
<td>24.02 ↓</td>
<td>168.166 ± 10.24***</td>
<td>47.88 ↓</td>
<td>14.83 ± 3.79</td>
<td>28.54 ↓</td>
</tr>
<tr>
<td>T. ciliata</td>
<td>135.16 ± 7.27***</td>
<td>35.13 ↓</td>
<td>174.833 ± 14.18***</td>
<td>42.17 ↓</td>
<td>168.666 ± 7.91***</td>
<td>44.72 ↓</td>
</tr>
<tr>
<td>B. spectabilis</td>
<td>99.166 ± 7.60***</td>
<td>30.26 ↓</td>
<td>193.00 ± 14.65***</td>
<td>46.00 ↓</td>
<td>168.00 ± 3.23***</td>
<td>46.26 ↓</td>
</tr>
<tr>
<td>F. glomerata (bark)</td>
<td>100.83 ± 5.42***</td>
<td>29.93 ↓</td>
<td>34.166 ± 3.94***</td>
<td>09.80 ↑</td>
<td>36.83 ± 0.910***</td>
<td>12.14 ↑</td>
</tr>
<tr>
<td>P. longifolia</td>
<td>107.5 ± 5.57***</td>
<td>29.68 ↓</td>
<td>115.833 ± 4.246***</td>
<td>37.78 ↓</td>
<td>139.5 ± 10.05***</td>
<td>42.60 ↓</td>
</tr>
<tr>
<td>F. glomerata (leaves)</td>
<td>118.666 ± 8.53***</td>
<td>37.27 ↓</td>
<td>195.50 ± 10.059***</td>
<td>46.88 ↓</td>
<td>150.833 ± 6.36***</td>
<td>46.94 ↓</td>
</tr>
</tbody>
</table>

↑ indicates increase locomotor activity and ↓ indicates decrease locomotor activity
Each value is the mean ± SEM of 6 determinations
***P < 0.001 dunnet test as compared to control (before treatment value)
Fig. 4.2

Effect of various plant extracts on locomotor activity score in rats as tested in actophotometer

- Chlorpromazine
- Caffeine
- S. acuta
- S. fruticosa
- T. ciliata
- B. spectabilis
- F. glomerata (bark)
- P. longifolia (leaves)
- F. glomerata (leaves)

Mean locomotor score

25mg

100mg/kg 300mg/kg 500mg/kg
4.6 Analgesic activity

4.6.1 Analgesic activity by Hot plate Method

One hundred and thirty eight rats, deprived of food (Water *ad libitum*) for 24 h. were randomly grouped into twenty-three groups, each consisting of 6 animals.

Group 1: received 1 ml/kg, i.p 12% Tween 80 as vehicle control

Group 2: received pentazocine 10 mg/kg, i.p as standard control (narcotic analgesic)

Group 3-5: received 100mg/kg, 300mg/kg, 500mg/kg, i.p of ethanolic extract of *S.acuta*

Group 6-8: received 100mg/kg, 300mg/kg, 500mg/kg, i.p of ethanolic extract of *S.fruticosa*

Group 9-11: received 100mg/kg, 300mg/kg, 500mg/kg, i.p of ethanolic extract of *T.ciliata*

Group 12-14: received 100mg/kg, 300mg/kg, 500mg/kg, i.p of ethanolic extract of *B.spectabilis*

Group 15-17: received 100mg/kg, 300mg/kg, 500mg/kg, i.p of ethanolic extract of *F.glomerata* (bark)

Group 18-20: received 100mg/kg, 300mg/kg, 500mg/kg, i.p of ethanolic extract of *P.longifolia*

Group 21-22: received 100mg/kg, 300mg/kg, 500mg/kg, i.p of ethanolic extract of *F.glomerata* (leaves)
The paws of mice and rats are very sensitive to heat at temperature, which are not damaging the skin. The response is in the form of jumping, withdrawal of the paws or the licking of the paws\textsuperscript{22, 23}. The animals were placed on Eddy’s hot plate kept at a temperature of 55±0.5°C. A cut off period of 15 sec., was observed to avoid damage to the paw. Reaction time and the type of response were using a stopwatch. The latency was recorded before and after 15, 30, 60 and 120 minutes following intraperitoneal administration of 100, 300 and 500 mg/kg of each of the extract to different groups of six animals each.

The analgesic activity data (Hot plate method) are presented in Table 4.3 and Fig. 4.3.
Table 4.3

Effects of various plant extracts administered intraperitoneally on the latency of rat exposed to the hot plate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>At 100 mg/kg</th>
<th></th>
<th>At 300 mg/kg</th>
<th></th>
<th>At 500 mg/kg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Latency</td>
<td>% Protection</td>
<td>Mean Latency</td>
<td>% Protection</td>
<td>Mean Latency</td>
<td>% Protection</td>
</tr>
<tr>
<td></td>
<td>time in (sec.) ± SEM</td>
<td></td>
<td>time in (sec.) ± SEM</td>
<td></td>
<td>time in (sec.) ± SEM</td>
<td></td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>2.167 ± 0.1667</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentazocine 10 mg/kg</td>
<td>6.833 ± 0.4014**</td>
<td>68.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. acuta</td>
<td>3.167 ± 0.3073</td>
<td>31.6</td>
<td>3.500 ±0.3416*</td>
<td>38.1</td>
<td>4.833 ± 0.477**</td>
<td>55.2</td>
</tr>
<tr>
<td>S. fruticosa</td>
<td>3.333 ± 0.2108</td>
<td>35.0</td>
<td>4.0 ± 0.3651**</td>
<td>45.8</td>
<td>4.667 ± 0.333**</td>
<td>53.5</td>
</tr>
<tr>
<td>T. ciliata</td>
<td>4.00 ± 0.2582**</td>
<td>45.8</td>
<td>4.167 ± 0.166**</td>
<td>48.0</td>
<td>6.333 ± 0.6146**</td>
<td>65.8</td>
</tr>
<tr>
<td>B. spectabilis</td>
<td>2.50 ± 0.2236</td>
<td>13.32</td>
<td>3.33±0.333</td>
<td>35.0</td>
<td>3.667 ± 0.210**</td>
<td>40.1</td>
</tr>
<tr>
<td>F. glomerata (bark)</td>
<td>2.667 ± 0.2108</td>
<td>18.74</td>
<td>3.33±0.2108</td>
<td>35.0</td>
<td>4.167 ± 0.48**</td>
<td>48.0</td>
</tr>
<tr>
<td>P. longifolia</td>
<td>2.333 ± 0.218</td>
<td>7.1</td>
<td>3.667±0.333**</td>
<td>40.1</td>
<td>4.33 ± 0.2108**</td>
<td>50.0</td>
</tr>
<tr>
<td>F. glomerata (leaves)</td>
<td>2.667± 0.333</td>
<td>18.74</td>
<td>3.667±0.333**</td>
<td>40.1</td>
<td>3.833 ± 0.3073**</td>
<td>43.5</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E.M of 6 determinations

**P < 0.01 dunnet test as compared to control
Fig 4.3

Effects of various plant extracts administered intraperitoneally on the latency time of rat exposed to the hot plate
4.6.2 Tail immersion method

Thirty mice of either sex deprived of food (Water *ad libitum*) for 24 h, were randomly grouped into five, each group consists of 6 animals

Group 1: received 1 ml/kg, i.p 12% Tween 80 as vehicle control

Group 2: received pentazocine 10 mg/kg, i.p as standard control (depressant)

Group 3-5: received 100mg/kg, 300mg/kg, 500mg/kg, i.p of ethanolic extract of *T.ciliata*

The procedure is based on the observation that morphine-like drugs are selectively prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55°C. The extract which showed maximum analgesic activity by hot plate method only was tested by tail immersion method to confirm its activity. The analgesic activity data (Tail immersion method) are presented in Table 4.4 and Fig.4.4
Table 4.4

Effect of ethanolic extract of *T.ciliata* (heartwood) administered intraperitoneally at doses of 100, 300 and 500 mg, tail withdrawal reflex of mice induced by tail immersion method.

Tail withdrawal reflex in seconds

<table>
<thead>
<tr>
<th>Treatment</th>
<th>After 30 min of drug treatment</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean reaction time ± SEM</td>
<td></td>
</tr>
<tr>
<td>Vehicle control</td>
<td>2.667 ± 0.2108</td>
<td>-</td>
</tr>
<tr>
<td>Pentazocine 10mg/kg</td>
<td>5.667 ± 0.2108**</td>
<td>52.94</td>
</tr>
<tr>
<td><em>T.ciliata</em> 100mg/kg</td>
<td>4.333 ± 0.333**</td>
<td>38.45</td>
</tr>
<tr>
<td><em>T.ciliata</em> 300mg/kg</td>
<td>7.000 ± 0.8944**</td>
<td>61.90</td>
</tr>
<tr>
<td><em>T.ciliata</em> 500mg/kg</td>
<td>4.833 ± 0.3073**</td>
<td>44.82</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM of 6 determinations

**P < 0.01 dunnet test as compared to control value.
Effect of ethanolic extract of T. ciliata administered intraperitoneally at doses of 100, 300 & 500 mg/kg, tail withdrawal reflex of mice induced by tail immersion method.

Fig. 4.4
4.7 Statistical analysis

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnets multiple comparisons using graph pad in stat 3 Demo and all the results obtained in the study were compared with the vehicle control group. P values <0.05 were considered statistically significant.

In case of locomotor activity the mean locomotor score was analysed by paired t-test, P<0.001 were considered statistically significant.

4.8 Results

The plant extracts *T.ciliata*, *B.spectabilis*, *F. glomerata* (bark, leaves) and *P.longifolia* at the dose of 100mg/kg, p.o, showed significant increase in mean immobility as compared to vehicle treated group (P<0.01). In case of *S.fruticosa* the increase in immobility was not significant. The results are shown in Table 4.1. One-way ANOVA showed that there is significant reduction in mean immobility time with *S.acuta* ethanolic extract at the dose of 100mg/kg and increase in swimming behavior (42.8 sec.), at higher dose level (300mg, 500mg/kg) the mean immobility time increases proportionately as compared to vehicle treated group (80.5,79.3sec. respectively).

On comparison of percentage change in activity among various plant extracts at 100mg/kg, p.o. it was observed that the degree of change in activity was greater with *B.spectabilis>* *T.ciliata>* *F.glomerata* (bark, leaves)> *P.longifolia>* *S.fruticosa*. (Table 4.1) Further the change in activity of these extract was comparable with that of standard drug Diazepam (Figure 4.1).

Locomotor activity was carried out by measuring motor activity using actophotometer. The mean locomotor scores of the different ethanolic extracts of the plant are shown in Table 4.2. Paired t-test indicates that significant change in the motor activity. After treatment with the extract the animal traversed more than pre-
treatment performance with *S. acuta*. The locomotor activity was found to increase with increase of the dose from 100mg to 300mg. Further increase of dose did not cause appreciable changes in activity, which indicated that optimum activity lies at 300mg/kg, p.o. In case of *F. glomerata* (bark) psychomotor stimulant activity was not seen at 100mg/kg (29.93%↓). But at 300mg and 500mg/kg the animal showed psychomotor stimulant activity. The other plant extract *S. fruticosa, T. ciliata, B. spectabilis, P. longifolia* and *F. glomerata* leaves showed decrease in motor activity (p<0.001). Increase of dose to a level of 300mg/kg showed significant decrease in motor activity. At 500mg/kg motor activity observed was negligible when compared to 300mg/kg. (Figure 4.2)

Various plant extracts when given in doses of 100mg, 300mg and 500mg/kg, i.p elicited a significant analgesic activity in the hot plate as evidenced by increase in latency time in seconds, (Table 4.3 and Figure 4.3) as compared with vehicle control at the end of 30 minutes. The increase in latency time was dose dependant. Latency time was noted at 0,15,30,60 and 120 minutes after the administration of vehicle, standard and plant extracts. Out of the seven plant extracts tested *T. ciliata*, at 100 mg/kg, i.p. showed significant increase in latency time at (P<0.01) (45.8% Protection), *S. fruticosa* significant at P<0.05 (35.0% Protection). The other plant extracts did not show any significant increase in the latency time.

At 300mg/kg, i.p. the following extract showed significant increase in latency time *T. ciliata, S. fruticosa, P. longifolia, F. glomerata* (leaves), (P<0.01) and percentage protection was found to be 48.0%, 45.8%, 40.1%, 40.1% respectively. But in *S. acuta, B. spectabilis*, and *F. glomerata* (bark), increase in reaction was significant at P<0.05. Percentage protection observed was 38.1, 35.0%, 35.0% respectively. At 500mg/kg i.p. all the extracts showed significant increase in reaction time. (P<0.01)
The tail withdrawal reflex time in *T.ciliata* ethanolic extract was found to be significant at 100mg/kg, 300mg/kg and 500mg/kg, i.p. in mice at 30 min. The effects of *T.ciliata* are comparable at 300mg/kg with that of standard pentazocine and in fact the percentage protection exceeds that of pentazocine. *(Table 4.4 and Fig. 4.4)*

4.9 Discussion

The results of this study showed that treatment of ethanolic extracts of the various plants have changed the active behavior of the animal in forced swimming test. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models are employed for evaluation of stress-precipitated behavior. FST in rats or mice has been widely used as an animal model to screen new antidepressant drugs. This test is specific to all major classes of antidepressant drugs including tricyclic antidepressant, serotonin specific re-uptake inhibitors and monoamino oxidase inhibitors\(^ {25,26}\). Antidepressant drug reduces immobility time. The treatment of different ethanolic extract of the plant *S.acuta* reduced the immobility time and increased swimming behavior at low dose (100mg/kg). This may be due to the presence of ephedrine as reported in literature. Increase of dose beyond this level resulted in decrease in the swimming behavior. There are reports to indicate that immobility; swimming and climbing behavior are enhanced by different groups of antidepressant drugs\(^ {15}\). The extracts of the other plants showed CNS depressant activity.

The change in motor activity with chlorpromazine is Blocking of DA D2 receptors in the mesolimbic-mesocortical system. The antipsychotic type activity of the plant extracts might be due to binding with the receptors\(^ {27}\). A receptor level further study might be needed to understand the mechanism behind it clearly.

The opioids have probably been studied more intensively than any other group of drugs in the effect to understand their powerful effects in molecular, biochemical
and physiological parameters. On the basis of the results opioid drugs could be developed as analgesics with significant advantages over morphine. While the receptor biology has been extensively studied the mechanism of action of analgesics has not been fully understood. Till date morphine remains the standard drug for any new analgesic tested.

Pentazocine mixed agonist antagonistic, in low doses acts very similar to morphine. It has a good binding with K-receptor than μ-receptor and it acts on non-opioid σ-receptor. Hence the use of pentazocine as a standard substitute for morphine in the present study.

The hot plate and tail immersion method is considered to be selective for opioid like compounds in several animal species\textsuperscript{28}. \textit{S.acuta} contains ecdysterone, ephedrine, hentriacontane, hypolaetin-8-glucoside; \textit{S.fruticosa} contains sterols and amino acids; \textit{T.ciliata} contains bergapten; \textit{B.spectabilis} contains pinitol a antidiabetic compound; \textit{F.glomerata} contains friedelin along with behanate, berganin, lupeol and its acetate; \textit{P.longifolia} contains quercetin, bulbocapnin. These chemicals may be responsible for norcotic analgesic, anti-inflammatory and antidiabetic activity. The steroids (β-sitosterol, stigmasterol and campesterol), which are common to these plants, may also contribute to the analgesic activity observed.
Structure of compounds possessing CNS activity (Antidepressant, Anti-psychotic and Analgesic properties)

- Ephedrine
- Cryptolepine
- Bergapten(e)
- β-sitosterol glycoside
- Stigmasterol glycoside
- β-Amyrin
- Friedelin
- Cedrelone
- Quercetin
- Bulbocapnine
4.10 References


