CHAPTER IV
Pharmacological Investigations of Olibergin-A Isolated from stem bark of *Dalbergia rostrata*

Flavonoids are being reported to possess a variety of biological activities and hence this group has gained much interest as active pharmacological agents recently. Flavonoids are known for their anti-inflammatory, antidiabetic, anti-allergic effects etc. They are also known for antithrombotic and vasoprotective properties, for inhibition of tumour promotion and as protectives for gastric mucosa. Many naturally occurring flavonoids are found to be diuretics or antispasmodics. Bae *et al.* have recently investigated the in-vitro anti helicobacter pylori activity of a number of flavonoids.

Szent-Gyorgyi* reported in 1938, a certain mixture of flavone and flavanone glycosides from *Capsicum annuum* and *Citrus limon* exhibiting prevention of capillary bleeding of scurvy. This mixture was later shown to consist of hesperidin, eriodictin and quercitrin*3*. With their reported activities hesperidin and eriodictin have been introduced in clinical practice to increase the capillary resistance. Similarly other flavonoids like hesperitin, lutin, quercetin, naringenin, kaempferol etc. have been identified and found to possess capillary resistance increasing potential*4*. Such developments clearly indicate the necessity and advantages of characterizing pure isolates from plant sources.

4.1 Present work

As a part of our study, we have investigated a few potential pharmacological activities of the crude extracts of *Dalbergia rostrata*,
Dalbergia malabarica and Derris benthamii and also an isolate from the alcoholic extract of stem bark of Dalbergia rostrata, 4’,5,7-tri-hydroxy-2’,5’-dimethoxyisoflavone (details of isolation and characterization are discussed already in Chapter II) for various pharmacological activities like antiinflammatory, hepatoprotective, antidiabetic, antioxidant, diuretic, antipyretic and analgesic, following the methodologies already discussed in Chapter III.

4.2 Anti-inflammatory Activity of Olibergin-A

Inflammatory reaction is readily produced in rats in the form of paw oedema with the help of inflammasens which are agents used to induce inflammation. Substances such as carrageenan, formalin, bradykinin, histamine, mustard and egg white. Any mechanism that leads to the inhibition of biosynthesis of the above substances is said to cause an anti-inflammatory response. In the present study, the test drugs are screened to identify their anti-inflammatory property.

4.2.1 Experimental Methodology

Three groups of six rats in each were taken for our study. The test drug Olibergin-A at a dose of 10 mg / kg was used. 0.1ml of the solution of 1% w/v of carrageenan was injected under the plantar region of the left paw according to the procedure described by Winter et al.\(^5\). The standard drug ibuprofen was used at a dose of 10 mg/kg body weight. It was injected 30 minutes prior to carrageenan challenge. The group administered with vehicle alone served as the control group. A Plethysmograph was used to record the paw volumes by the displacement of mercury in the column. The marks on
both the hind paws were made just beyond the tibio-tarsal junction to note the paw volumes.

The paw volumes of the control, Olibergin-A treated group and the standard drug groups were noted at 30, 60, 120, 180, 240, 300 and 360 minutes after carrageenin challenge. The difference between the right and left paw volumes corresponded to the oedema volume and the percentage of oedema inhibition by comparison with the control groups was calculated by using the formula as shown in Chapter III. The experimental observation and the percentage inhibitions in paw oedema volumes are given in Table 4.1 and Table 4.2 respectively.

**Table 4.1 Anti-inflammatory activity of Olibergin-A**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg body weight</th>
<th>paw oedema volumes (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>1.00 ± 0.25</td>
</tr>
<tr>
<td>Standard</td>
<td>10</td>
<td>0.544 ± 0.22**</td>
</tr>
<tr>
<td>Olibergin-A</td>
<td>10</td>
<td>0.33 ± 0.21*</td>
</tr>
</tbody>
</table>

ANOVA F - values * P<0.05 **P<0.01
Student’s t-test *P<0.05 **P<0.02 ***P<0.01 n.s : not significant

**Table 4.2 Anti-inflammatory activity of Olibergin-A**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose of the drug mg/kg body weight</th>
<th>Paw oedema volumes (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60 min</td>
</tr>
<tr>
<td>Standard</td>
<td>10</td>
<td>58.50</td>
</tr>
<tr>
<td>Olibergin -A</td>
<td>15</td>
<td>58.50</td>
</tr>
</tbody>
</table>

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4.2.2. Results and discussion

From Tables 4.1 and 4.2, it is seen that the onset of the activity of the standard drug ibuprofen was observed at 60 minutes after drug administration. The standard drug showed significant reduction in the paw volumes as compared to the untreated control group indicating that the model employed for the present study is appropriate and the data thus obtained could be tested for their significance of anti-inflammatory activity of the different test drugs.

From Table 4.1, it is observed that Olibergin-A at a dose of 10 mg/kg showed an early onset activity, which is significant (P<0.05) when compared to the untreated control group.

All the test groups were tested for their level of significance with respect to the control group using Dunnett’s t-test. The statistical method of one way ANOVA was applied for observations between time intervals of 1 hour to 6 hours after drug administration. The data
compared to the control group, with the maximum significance observed after a time interval of 2 hours after drug administration (Fig. 4.1). The percentage inhibition in the paw oedema volume up to 6 hours was noted for finding the duration of the anti-inflammatory activity.

It is observed from Table 4.2 that very good inhibition in paw oedema volumes that is comparable with that of the standard drug ibuprofen from 2 to 6 hours after drug administration is exhibited by Olibergin-A.

The significant anti-inflammatory activity of Olibergin-A may be due to either inhibition of prostaglandin biosynthesis on inhibition of platelet aggregation (induced by arachidonic acid) or due to their effect on histamine release, lipoxygenase and leucotriene synthesis.

4.3 Hepatoprotective activity of Olibergin-A

Liver is an important organ for the detoxification of endogenous and exogenous substances. Its function may readily be impaired by viruses, hepatotoxins and xenobiotics. The functional integrity of liver can be assessed by measuring the enzyme levels, bile flow and bile pigments. CCl4, a well known model to induce hepatic injury, requires biotransformation by hepatic microsomal P450S to produce the hepatotoxic metabolite, trichloromethyl radical. Management of hepatic damage is still a major problem in developing countries as the drugs used for it are either not readily available or expensive and ineffective. The hepatoprotective activity of Olibergin-A isolated from stem bark of Dalbergia rostrata was evaluated in albino rats.
4.3.1 Experimental methodology

The method of Anand et al. was followed for evaluation of hepatoprotective activity of Olibergin-A. The animals were divided into three batches, each consisting of three groups of six animals each. The treatment protocol given is as follows:

In all the batches, group I was kept as normal control, group II as CC\textsubscript{14} control, group III was treated orally with Olibergin-A at a dose level of 10mg/kg. CC\textsubscript{14} (0.75 ml/kg) mixed with an equal volume of liquid paraffin was administered orally by gastric intubation. The animals of group III were treated with 10 mg/kg of Olibergin-A 2 hours before CC\textsubscript{14} intoxication. Blood was collected from animals of all the groups 18 hours after CC\textsubscript{14} administration and serum was estimated for AST, ALT, ALP, serum bilirubin and total proteins\textsuperscript{13}. The results are tabulated in Table 4.3.

| Table 4.3 Effect of Olibergin-A on CCl\textsubscript{4} induced hepatotoxicity in rats |
|-----------------------------------------------|------------|------------|-------------|-----------------|-----------------|-----------------|
| Group Treatment                              | AST U/l    | ALT U/l    | ALP U/l     | Total Bilirubin mg% | Total Protein g% | Liver weight g |
| Group I Control                              | 138.2 ± 2.1| 47.2 ± 1.8 | 180.7 ± 5.2 | 0.69 ± 0.02         | 9.43 ± 0.31      | 3.80 ± 0.08    |
| Group II Positive control                     | 279.8 ± 1.8** | 101.2 ± 2.9*a | 395.6 ± 8.6*a | 1.30 ± 0.03*a      | 9.17 ± 0.28      | 7.10 ± 0.33**  |
| Group III Olibergin A                        | 158.2 ± 1.3*b | 65.4 ± 2.1*b | 275.3 ± 5.1*b | 1.93 ± 0.08*b      | 10.11 ± 0.32     | 6.15 ± 0.30*b  |

Newman keuPs multiple range test was used (*P<0.05)
*a: different from Group I *b: different from Group II
4.3.2 Results and discussion

Liver tissue is rich in transaminases, increased profoundly after acute hepatic liver injury and elevation of AST is an excellent marker of liver diseases. Elevation of other biochemical parameters also indicates a damaged liver. After the treatment with the extracts, all the enzyme levels were reduced towards normalcy. Bile is synthesized in liver and concentrated in gall bladder. Estimation of bile pigments adds support to the extent of liver injury. In our study, following CC1₄ administration, there was a significant increase in total bilirubin which was reduced to near normal values after administration of Olibergin-A. However, the protein content was not altered by CC1₄ treatment.

Further the results of various biochemical parameters was confirmed by the histopathological studies. Multiple section studies on the liver of the normal group (treated with vehicle alone) showed preservation of normal lobular architecture of the liver (Plate 4.1). The biopsy study of the liver intoxicated with CCI₄ (Plate 4.2) indicated the distortion of lobular architecture of the organ. From Plate 4.3, it was observed that Olibergin-A recovered fatty changes caused by CC1₄ and almost normalized hepatocytes.

Trichloromethyl, a free radical formed from CCl₄ is responsible for hepato-toxicity. This free radical initiates lipid peroxidation which further induces cell damage and as a result, the levels of all enzymes are elevated. AST level, though increased in cardio-necrosis is more related to liver damage and it is released into the blood stream in large amounts. Olibergin-A maintained the integrity of liver cell membrane as evidenced by significant reduction of AST and ALT.
Plate 4.1 Normal lobular architecture of liver

Plate 4.2 Distorted lobular architecture of liver due to CCl₄ intoxication

Plate 4.3 Recovered lobular architecture of liver due to administration of Olibergin-A
levels hiked by CCl₄ administration. Reduction in serum bilirubin indicated the restoration of the functional status of liver. The increase in liver weight after CC₁₄ treatment is due to its enlargement. The test drug Olibergin-A was able to reduce the enlarged liver showing significant hepatoprotective activity.

4.4 Antidiafoetic activity of Olibergin-A

Various agents like alloxan, diazoxide and streptazotocin are used to induce diabetes in experimental animals. These models are used to screen antidiabetic activity of biologically active constituents and in the present study, alloxan induced diabetic rat models were used to evaluate the antidiabetic activity of Olibergin-A isolated from stem bark of *Dalbergia rostrata*.

4.4.1 Experimental methodology

The antidiabetic activity was evaluated by the method followed by Kameswara Rao *et al*. The animals were fasted for 24 hours and injected with alloxan (150 mg/kg) intraperitoneally. After seven days, the rats were used for antidiabetic study. The rats with more than 300 mg/kg fasting blood glucose were selected and were divided into three batches, each consisting of five groups of six animals each. The treatment protocol is as follows:

In each batch, group I was reserved as diabetic control and group II was treated with tolbutamide (10 mg/kg) p.o. as standard drug. Group III was given Olibergin-A at a dose of 10 mg/kg p.o.
Blood samples, collected from the individual groups of animals at two different intervals of time (5th & 10th hour) after treatment with Olibergin-A and tolbutamide were analyzed for blood glucose. The results are tabulated in Table 4.4.

Table 4.4 Effect of Olibergin-A on blood glucose levels of alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Serum glucose at intervals of time mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Group I Diabetic control</td>
<td>309.0 ± 1.2</td>
</tr>
<tr>
<td>Group II Standard</td>
<td>323.0 ± 2.1</td>
</tr>
<tr>
<td>Group III Olibergin-A</td>
<td>212.5 ± 3.2</td>
</tr>
</tbody>
</table>

Newman keul’s multiple range test was used

*significantly different from zero hour reading (*P<0.05)

4.4.2. Results and Discussion

It is evident that Olibergin-A was able to lower the elevated blood glucose level at the 5th & 10th hour of administration. However,
the hypoglycemic effect for Olibergin-A was found to be less when compared with that of the standard drug tolbutamide (Fig. 4.2).

Alloxan induces diabetes by causing irreversible damage to the pancreas. It acts in three phases; an initial raise, a transient fall followed by a prolonged increase in the blood sugar levels. After 7 days, all the rats became sufficiently hyperglycemic to be used as models for evaluation of antidiabetic activity. Olibergin-A treated rats when evaluated after one week of administration of alloxan, showed moderate antidiabetic effect on prolonged hyperglycemic phase.

4.5 Antioxidant activity of Olibergin-A

Flavonoids are shown to possess pronounced antioxidant activity. Flavonoids occurring in fresh pepper, other vegetables and fruits, tea, soya bean, *Glycyrrhiza inflata*, stem bark of *Curdrania tricuspidata*™, young green barley leaves and *Corylus columbia* leaves have strong antioxidant activity and hence the test compound Olibergin-A isolated from the alcoholic extract of the stem bark of *Dalbergia rostrata* has been screened for its antioxidant activity.

4.5.1 Experimental methodology

The free radical scavenging effect has been studied for the test compound. In this study, the animals were divided into 3 groups of six each, designated as group I, group II and group III. The treatment protocol followed is as given below:
Group I was kept as negative control, group II as alcohol fed positive control with induced peroxidative liver damage and group II] treated with the Olibergin-A (10 mg/kg) suspended in 1% CMC p.o. The method followed by Mahendran and Shyamaladevi\textsuperscript{24}, as described in Chapter III has been followed to evaluate the free radical scavenging effect of the test compound. The results are shown in Table 4.5.

**Table 4.5 Effect of Olibergin -A against ethanol induced liver damage in rats**

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Total Cholesterol (mg %)</th>
<th>Triglycerides (mg %)</th>
<th>AST (U/I)</th>
<th>ALT (U/I)</th>
<th>GSH (mmol/g tissue)</th>
<th>CP (mmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Control</td>
<td>63.71 ± 0.68</td>
<td>128.94 + 1.58</td>
<td>142.29 + 1.04</td>
<td>53.84 ± 1.21</td>
<td>5.11 ± 0.03</td>
<td>168.2 ± 2.4</td>
</tr>
<tr>
<td>Group II Positive control</td>
<td>84.4 + 3.6*\textsuperscript{a}</td>
<td>185.0 ± 5.1*\textsuperscript{a}</td>
<td>298.0 ± 4.3 *\textsuperscript{a}</td>
<td>100.21 ± 1.0*\textsuperscript{a}</td>
<td>3.22 ± 0.0*\textsuperscript{a}</td>
<td>249.0 ± 4.6*\textsuperscript{a}</td>
</tr>
<tr>
<td>Group III Olibergin- A</td>
<td>71.4 ± 1.9\textsuperscript{b}</td>
<td>139.0 ± 2.8 *\textsuperscript{b}</td>
<td>160.2 ± 7.0*\textsuperscript{b}</td>
<td>75.4 ± 2.9*\textsuperscript{b}</td>
<td>4.82 ± 0.2*\textsuperscript{b}</td>
<td>162.0 ± 2.7*\textsuperscript{b}</td>
</tr>
</tbody>
</table>

*\textsuperscript{a} different: from group I *\textsuperscript{b} different from group II
Newman keul's multiple range test was used (*P<0.05)

Fig.4.3. Antioxidant activity of Olibergin-A
4.5.2 Results and Discussion

Chronic administration of alcohol to the animals in group II resulted in abnormal rise in total cholesterol and triglycerides. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glutathione peroxidase (GP) were also elevated and glutathione was depleted indicating liver cell damage. Treatment with the test compound reduced the levels of lipid, enzyme and glutathione peroxidase but elevated the reduced glutathione (GSH).

Continuous administration of ethanol induced hyperlipidemia that results in liver damage by elevating the levels of enzymes and lowering the glutathione (GSH). Glutathione, a tripeptide plays a major role in the protection of cells and tissues. Chronic administration of alcohol resulted in depletion of GSH which was elevated in the test compound treated group, suggesting the restoration of balance between depletion and formation. Glutathione peroxidase catalyses the oxidation of GSH. A reduced glutathione peroxidase activity was observed in the test compound treated group (Fig.4.3). Probably through this mechanism, the test compound might be restoring the GSH levels indicating that it possesses free radical scavenging activity.

4.6 Diuretic activity of Olibergin-A

Diuretics are agents that increase the rate of urine formation. They are commonly used in edema and also in cardiovascular disorders, particularly in hypertension, where mild 'diuretics are preferred. In the present study, Olibergin-A from Dalbergia rostrata has been screened for its diuretic activity.
4.6.1 Experimental methodology

The method of Lipschitz\textsuperscript{24} was followed to evaluate the diuretic activity. Male albino Wistar rats, each weighing 150-200 g selected for the study were divided into three batches, each consisting of three groups of six animals each. The treatment protocol followed is as given below:

Group I was kept as normal control and group II was given the standard drug frusemide and group III received a dose of 10 mg/kg of Olibergin-A respectively.

The animals were fasted for 18 hours prior to the experiment. Control group received 0.5 ml of normal saline followed by 2 ml of 1\% CMC suspension. The other groups were treated with normal saline followed by the Olibergin-A at a dose of 10 mg/kg suspended in 2 ml of 1\% CMC orally. The standard group was treated with frusemide (5 mg/kg p.o.). The groups of animals were placed individually in metabolic cages and urine was collected during the next 12 hours. The volume of urine was noted and analyzed for sodium, potassium, chloride and bicarbonate ions. Sodium and potassium ions were estimated by flame photometry and chloride and bicarbonate ions by titrimetry. The results are shown in Table 4.6 and in Fig 4.4.
Table 4.6 Diuretic potential of Olibergin-A

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Volume of urine (ml)</th>
<th>Na⁺ (meq/L)</th>
<th>Cr (mmol/L)</th>
<th>HCO₃⁻ (mmol/L)</th>
<th>K⁺ (meq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Control</td>
<td>3.10 ± 47.0</td>
<td>64.0 ± 2.4</td>
<td>84.0 ± 1.47</td>
<td>12.2 ± 1.11</td>
<td>14.48 ± 3.71</td>
</tr>
<tr>
<td>Group 11 Standard</td>
<td>6.73 ± 24.0*&quot;</td>
<td>440.0 ± 3.41**</td>
<td>145.01</td>
<td>24.31</td>
<td>15.20 ± 4.11*&quot;</td>
</tr>
<tr>
<td>Group III</td>
<td>3.90 ± 1.21*&quot;</td>
<td>80.0 ± 4.31*&quot;</td>
<td>89.01</td>
<td>3.5as*</td>
<td>14.73 ± 5.1*&quot;</td>
</tr>
</tbody>
</table>

Newman keul’s multiple range test was used ("P< 0.05)
*a - different from Group I
*b - different from Group II
n.s. - not significant

Fig.4.4. Diuretic activity of Olibergin-A

4.6.2. Results and Discussion

The test compound Olibergin-A somewhat increased the volume of urine followed by nominal increase in excretion of chlotide and bicarbonate ions. The volume of urine and excretion of sodium, potassium chloride and bicarbonate ions by Olibergin-A are different from control group.
Olibergin-A enhances the urine volume but not that much as frusemide. The increase in sodium and potassium ion excretion showed that the Olibergin-A produce natriuretic and kaliuretic effects though not significant. In our study, the chloride ion excretion is also observed to be slightly high. As the standard dmg employed is a high ceiling diuretic, Olibergin-A was found to induce diuresis to some extent though not comparable to that of the standard.

4.7. Antipyretic activity of Olibergin-A

During fever, body temperature is set at a higher point and antipyretics reduce this, thereby lower the elevated body temperature. Most of the antipyretics reduce the elevated body temperature and do not alter the normal body temperature. Hence antipyretics are evaluated by using animal models in which the body temperature is set at a higher point by the injection of brewer’s yeast.

4.7.1, Experimental methodology

The antipyretic activity of Olibergin-A was studied against yeast-induced pyrexia by following the method of Smith and Hambourger\(^{26}\). Male Wistar rats were divided into three groups, each consisting of six animals. The treatment protocol followed is as given below:

Group I was kept as pyretic control, Group II received the standard drug aspirin, and Group III treated with Olibergin-A.. The basal temperature was recorded using a rectal telethermometer and 15 % w/v of yeast suspended in 0.05% w/v CMC solution was subcutaneously injected at a dose of 10 ml/kg body weight.
Olibergin-A was given orally at a dose of 10 mg/kg p.o. after 10 hours of the injection of yeast and the standard drug aspirin was administered orally at a dose of 10 mg/kg. The body temperature was recorded after 5th and 10th hour. The results are tabulated in Table 4.7.

<table>
<thead>
<tr>
<th>Group/ Treatment</th>
<th>Body temperature ( °C)</th>
<th>Before yeast injection</th>
<th>After drug administration</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sh</td>
<td>10 h</td>
<td>10 h</td>
<td></td>
</tr>
<tr>
<td>Group I Pyretic control</td>
<td>37.6 ± 0.03</td>
<td>39.8 ± 0.03</td>
<td>39.7 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II Standard</td>
<td>37.4 ± 0.04</td>
<td>37.6 ± 0.02*</td>
<td>37.5 ± 0.03*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III Olibergin-A</td>
<td>37.9 ± 0.02</td>
<td>38.1 ± 0.03*</td>
<td>38.4 ± 0.01*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 Newman keul’s multiple range test was used.

4.7.2. Results and Discussion

It was found that Olibergin-A at a dose of 10 mg/kg, lower body temperature moderately after 5 hours of administration. Olibergin-A showed a decrease in body temperature which is not comparable with the standard drug.

In the present study, a very minimum reduction in the body temperature was observed with the compound. Screening of the compound for their analgesic activity showed them to possess analgesia as compared with the standard drugs used. In our study, the compound exhibited some antipyretic effect in addition to the analgesic effect though not comparable to that with the standard aspirin.
4.8. Analgesic activity of Olibergin-A

Analgesics are drugs, which relieve pain. Separate methods are available to study peripheral analgesics and central analgesics. In this study, hot plate method and caudal compression methods are used to evaluate the analgesic effect of Olibergin-A. In both the analgesic methods, the latency between the application of obnoxious stimuli and the response was considered as the ‘basal reaction time’.

4.8.1 Experimental methodology

4.8.1.1. Hot Plate method

The modified method of Eddy et al.\textsuperscript{27} was followed to study the analgesic effect. Swiss albino mice weighing 25-30 g each, selected for the study were divided into three groups of six animals each. The treatment protocol followed is as follows:

Group I kept as normal control, received 2 ml of 1% CMC and group II received the standard drug pentobarbitone at a dose of 4.0 mg/kg i.p. and group III administered with Olibergin-A at a dose of 10mg/kg p.o.

The animals were placed on Eddy’s hot plate maintained at 55°C and their response by paw licking and jumping was observed. The basal reaction time for the animals of all the groups was recorded initially and the groups were given the respective treatment. The latency was measured at 30 min. intervals up to 2 hours and the results are shown in Table 4.8 and Fig. 4.5.
Table 4.8 Analgesic activity of Olibergin-A by Hot plate method

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Basal reaction time after drug administration (sec) mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Control</td>
<td>3.10 ± 0.25 3.30 ± 0.30 3.60 ± 0.21 3.50 ± 0.35 3.60 ± 0.40</td>
</tr>
<tr>
<td>Group II Standard</td>
<td>3.40 ± 0.15 12.30 ± 0.25 12.70 ± 0.25* 12.10 ± 0.44* 11.20 ± 0.52*</td>
</tr>
<tr>
<td>Group III Olibergin A</td>
<td>3.20 ± 0.10 8.30 ± 0.42* 10.10 ± 0.25* 9.50 ± 0.35* 8.60 ± 0.51*</td>
</tr>
</tbody>
</table>

*P< 0.05 Newman keul’s multiple range test was used

Fig.4.5. Analgesic activity of Olibergin-A (Hot plate method)

The latency between the noxious stimulus and the response was prolonged by pentazocine and Olibergin-A after half an hour of their administration. However the latency was continuing to be normal in group I throughout the experiment.

4.8.L2. Caudal compression method

The method of Bianchi et al.\textsuperscript{28} was followed. Swiss albino mice, each weighing 25-30 g, selected for the study were randomly divided into three groups of six animals each. The treatment protocol followed is as given below:
Group I received 2 ml of 1% CMC and kept as normal control and group II received the standard drug pentazocine at a dose of 4.0 mg/kg i.p. and group III was given Olibergin-A at a dose of 10 mg/kg p.o.

An artery clip was applied to the base of the tail for 30 seconds and the response of the animals for the removal of clip was noted. The basal reaction time of the animals of all groups was recorded initially and the groups were given respective treatment with Olibergin-A. The latency was measured at an interval of 30 min upto 2 hours. The results are shown in Table 4.9 and Fig.4.6.

<table>
<thead>
<tr>
<th>Group/ Treatment</th>
<th>Basal reaction time after drug administration (sec) mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Group I Control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.70 ±</td>
</tr>
<tr>
<td>Group II Standard</td>
<td>3.60 ±</td>
</tr>
<tr>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>Group III Olibergin-A</td>
<td>3.50 ±</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
</tr>
</tbody>
</table>

* P < 0.05 Newman keul’s multiple range test was used
*a - different from 0 min. reading in the respective group.
The basal reaction time for normal control was found to be between 2.7 and 3.0 min. However this latency was found prolonged by treatment with standard drug at 30 min. after administration and the maximum prolongation of latency was seen after 120 min. for Olibergin-A.

4.8.1.3. Results and Discussion

Though many methods are available to evaluate analgesic activity, Eddy’s hot plate method is commonly used to evaluate centrally acting analgesics. Olibergin-A was found to prolong the basal reaction time significantly in the hot plate method and to a lesser degree in the caudal compression method as compared to the standard drug. In both the methods, the analgesia produced was slightly lesser than that of the standard pentazocine. However in the caudal compression model, Olibergin-A showed an effect lasting upto the 120th min. of study whereas with pentazocine, the effect started fading after 90th min. It is perhaps because Olibergin-A acts through peripheral mechanism as well.
References


