CHAPTER IV

Pharmacological investigations of *Dalbergia rubiginosa* and *Dalbergia malabarica*

Flavonoids, a group of phenolic derivatives with diverse chemical structures are biosynthesized in substantial amounts, nearly upto 1.5% in a wide variety of plants\(^1\). Flavonoids have gained much interest as bioactive principles since a variety of biological activities of this group of natural products has been reported of late\(^2\)-\(^8\). A copious availability of reference materials as discussed in Chapter-I indicates the extent of pharmacological work being carried out on *Dalbergia* species.

*Dalbergia*, a genus of Leguminosae with about 120 species, has been a source of a large number of compounds including flavonoids and isoflavonoids\(^9\). A number of reviews have been reported on the chemical constituents of *Dalbergia*\(^10\)-\(^13\).

*Dalbergia* species that have been exhaustively studied and reported for varied pharmacological activities include *D. sissoo*\(^14\), *D. lanceolaria*\(^14\), *D. nigra*\(^14\), *D. parviflora*\(^14\) and *D. variabilis*\(^14\), *D. volubilis*\(^15\), *D. odorifera*\(^16\), *D. monetaria*\(^\text{v}\), *D. lattifolia*\(^\text{v}\), *D. hancei*\(^19\), *D. sissoides*\(^20\), *D. cochinchinensis*\(^21\), *D. candenatensis*\(^\text{v}\) etc. The activities observed include antiarthritic, anti-inflammatory, antifungal, antibiotic, haematinic, antilipidemic, antitumor, antiandrogenic etc.

4.1 Present work

In this study, two species of *Dalbergia* viz. *Dalbergia rubiginosa* Roxb and *Dalbergia malabarica* Prain, both not hitherto pharmacologically screened have been taken up for investigation. The
crude extracts of both *D. rubiginosa* and *D. malabarica* have been screened for antioxidant, antilithiatic, antifibrotic, hepatoprotective, antidiabetic, diuretic, anti-inflammatory, antipyretic and analgesic activities. The results subjected to suitable statistical analysis for veracity are presented.

4.2 General methodology

The general experimental conditions maintained and the methods carried out for pharmacological screening are as follows:

Male Wistar strain albino rats, weighing around 150-200 g each, were procured from the animal house of Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli (Regd.No. 265/CPCSEA). The mean age of the animals was 6-8 weeks. Animals were acclimatized to our laboratory conditions for atleast 3 days before induction into experiments. The animals were then selected at random to form groups of six each and were identified by conventional type of individual markings on tails, limbs and heads and also by group numbers.

Each group of animals, housed separately in polypropylene cages provided with husk bedding was fed with standard laboratory feed pellets supplied by Amrut Pranav Ltd., Bangalore and fresh filtered tap water *ad libitum*. The house temperature was maintained at 25 ± 2° C with relative humidity of 50-70% and 12 hours Light/Dark cycles. The animals were fasted for 16 hours before and 2 hours after the administration of the extracts.

The routes of drugs administration were either oral or intraperitoneal using 1% carboxymethylcellulose (CMC) at a dose of
1 ml/kg p.o. unless otherwise specified. The dose of test samples used was 100 mg/kg, selected on the basis of the report of LD₅₀ for the ethanolic extract of *D. rubiginosa* in mice. This dose determination was made on the basis of the toxicological work on *D. rubiginosa* by Bhakuni *et al*²².

4.3 Antioxidant activity

Free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation.²³ Antioxidants of natural origin can protect the human body from free radical induced injury²⁴. Flavonoids have been reported to possess pronounced antioxidant effect and hence a large number of medicinal plants with flavonoids as their phytoconstituents are being screened for their free radical scavenging effect²⁵.

4.3.1 Methodology

The method followed by Mahendran and Shyamaladevi²⁶ has been adopted to evaluate the free radical scavenging effect of the extracts of leaves of *D. rubiginosa* (DRL) and *D. malabarica* (DML). The animals were divided into two batches, each consisting five groups of six animals each, designated as G1 through G5 and the treatment protocol followed is as follows:

In each batch, G1 was reserved as negative control; peroxidative liver damage was induced in G2, G3, G4 and G5 by feeding the animals with ethanol. G2 was reserved as alcohol fed positive control and G3, G4 & G5 were treated with acetone, alcohol and aqueous extracts of DRL and DML in their respective batches.
Peroxidative liver damage was induced in G2 by feeding the rats with 18% ethanol for 45 days (5 ml/100 g). The groups G3, G4 and G5 were simultaneously treated with \textit{100} mg/kg of acetone, alcohol and aqueous extracts of DRL and DML orally in their respective batches. On day 45, the overnight fasted rats were anaesthetized and blood was collected by carotid artery bleeding. Serum was analyzed for triglycerides, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutathione peroxidase (GP), reduced glutathione (GSH) by using auto analyzer. The results are shown in Table-4.1 & Table-4.2 and presented in Fig. 4.1, 4.1.1 & Fig. 4.2, 4.2.1 for DRL and DML respectively.

Table-4.1 Effect of DRL 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/l)</th>
<th>ALT (U/l)</th>
<th>GSH (mmol/g tissue)</th>
<th>GP (mmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Control</td>
<td>65.82 ± 0.79</td>
<td>126.8 ± 1.57</td>
<td>140.1 ± 1.04</td>
<td>51.72 ± 1.18</td>
<td>4.50 ± 0.01</td>
<td>170.0 ± 2.1</td>
</tr>
<tr>
<td>G2 Positive control</td>
<td>88.51 ± 1.35* a</td>
<td>178.1 ± 1.02* a</td>
<td>272.3 ± 1.62* b</td>
<td>96.18 ± 0.89* a</td>
<td>3.40 ± 0.03* a</td>
<td>242.0 ± 1.8* a</td>
</tr>
<tr>
<td>G3 Acetone extract</td>
<td>71.4 ± 1.1* b</td>
<td>165.0 ± 2.1* b</td>
<td>156.0 ± 2.7* b</td>
<td>59.40 ± 2.4* b</td>
<td>4.30 ± 0.02* b</td>
<td>163.0 ± 1.6* b</td>
</tr>
<tr>
<td>G4 Alcoholic extract</td>
<td>70.21 ± 0.88* b</td>
<td>141.79 ± 0.91* b</td>
<td>152.17 ± 1.61* b</td>
<td>58.53 ± 1.33* b</td>
<td>4.20 ± 0.04* b</td>
<td>165.0 ± 1.4* b</td>
</tr>
<tr>
<td>G5 Aqueous extract</td>
<td>81.0 ± 1.1* b</td>
<td>171.0 ± 3.1* b</td>
<td>166.0 ± 2.4* b</td>
<td>83.1 ± 4.41* b</td>
<td>4.40 ± 0.02* b</td>
<td>169.0 ± 2.2* b</td>
</tr>
</tbody>
</table>

*a different from G1  *b different from G2

Newman keul’s multiple range test was used (*P<0.05)
Effect of DRL against ethanol induced liver damage in rats

Fig. 4.1

Fig. 4.1.1
Table-4.2 Effect of DML 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/l)</th>
<th>GSH (mmol/g tissue)</th>
<th>GP (mmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Control</td>
<td>65.8 ± 0.79</td>
<td>126.8 + 1.57</td>
<td>140.1 ± 1.04</td>
<td>51.7 ± 1.18</td>
<td>4.50 ± 0.01</td>
<td>170.0 ± 2.1</td>
</tr>
<tr>
<td>G2 Positive control</td>
<td>88.51 ± 1.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>178.1 ± 1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>272.3 ± 1.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.18 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.40 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>242 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3 Acetone extract</td>
<td>79.0 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>177.0 ± 3.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>139.0 ± 3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.0 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.90 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>173.0± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4 Alcoholic extract</td>
<td>84.0 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>189.0 ± 5.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>300.0 ± 5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.0 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.0 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>181.0 ± 3.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G5 Aqueous extract</td>
<td>80.0 ± 2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>163.0 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>189.0 ± 4.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.0 ± 9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.1 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>179.0 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*a different from G1  *b different from G2

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

4.3.2 Results

Chronic administration of alcohol to the animals in G2 (positive control) resulted in abnormal raise in total cholesterol and triglycerides above the normal levels. The serum levels of the enzymes AST and ALT were also found increased after ethanol administration. Reduced glutathione was depleted and glutathione peroxidase level was elevated. Simultaneous administration of the alcoholic, acetone and aqueous extracts of DRL reduced the lipid, enzymes and glutathione peroxidase levels; but elevated the GSH. However maximum reduction was noted with the group treated with the alcoholic extract of DRL. Likewise, simultaneous administration of alcoholic, acetone and aqueous extracts of DML reduced the lipid, enzyme and glutathione peroxidase levels and elevated the GSH. Between the two Dalbergia species taken for our
Effect of DML against ethanol induced liver damage in rats

Fig. 4.2

![Graph showing various liver function tests](image)

Fig. 4.2.1

![Graph showing GSH levels](image)
study, the extracts of DRL exhibited pronounced antioxidant activity than that of DML.

4.33 Discussion

Ethanol on continuous administration, induces hyperlipidemia that results in liver damage by increasing the levels of enzymes and lowering the glutathione (GSH). Glutathione is a tripeptide, which plays a major role in the protection of cells and tissues. Low concentration of GSH has been observed in alcohol treated group, which is sufficient to indicate GSH depletion. GSH depletion normally follows a non-linear kinetics as with chronic paracetamol toxicity. In this study also, chronic alcohol administration resulted in GSH depletion and GSH level, found elevated in the extract treated groups suggested the restoration of balance between depletion and formation. Glutathione peroxidase catalyses the oxidation of GSH. A reduced glutathione peroxidase activity was observed in the extract treated groups. Probably the test extracts might be restoring the GSH levels through this mechanism and as the extracts have shown improved GSH levels, it is concluded that the test extracts possess free radical scavenging activity.

4.4 Antilithiatic activity

Urolithiasis is the third most common afflictions found in humans. At present the management of urolithiasis mainly involves the surgical removal of stones. Three theories have been proposed to explain the occurrence of lithiasis. Common principle of these theories is either supersaturation of ions or deficiency of inhibitory ions in urine that leads to stone formation. Thus changes in ionic pattern of urine results in lithiasis. Ethylene glycol was used to induce changes in the ionic pattern
of urine and hence lithiasis in rats and the antilithiatic effect of extracts of leaves of *D. rubiginosa* (DRL) and *D. malabarica* (DML) was studied.

4.4.1 Methodology

The antilithiatic activity was evaluated by using the method followed by Christina *et al.*

Male albino rats acclimatized to our laboratory conditions were divided into two batches, each having four groups of six animals each, designated as G1 through G4. In both the batches, G1 was reserved as normal control. Lithiasis was induced in G2, G3 and G4 by feeding the animals with 1% ethylene glycolated water for 35 days. G2 was reserved as lithiatic control. G3 and G4 were treated with 100 mg/kg of the alcoholic and aqueous extracts of DRL and DML suspended in 1% CMC orally. On day 34, the animals were kept in metabolic cages and urine was collected for 24 hours on the 35th day. The volume of urine was noted and analyzed for calcium, magnesium, oxalate, phosphate and proteins. The results are shown in Table-4.3 & Table-4.4 and presented in Fig. 4.3 & Fig. 4.4 for DRL and DML respectively.
### Table-43 Inhibitory effect of BRL on ethylene glycol induced hyperoxaluria

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Vol of urine ml/24 h</th>
<th>Calcium mg/24 h</th>
<th>Oxalate mg/24 h</th>
<th>Phosphate mg/24 h</th>
<th>Magnesium mg/24 h</th>
<th>Protein mg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Control</td>
<td>5.30 ± 0.2</td>
<td>0.59 ± 0.29</td>
<td>0.38 ± 0.02</td>
<td>5.90 ± 0.52</td>
<td>0.89 ± 0.06</td>
<td>2.78 ± 0.39</td>
</tr>
<tr>
<td>G2 Lithiatic control</td>
<td>2.90 ± 0.3*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.60 ± 0.34*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.20 ± 0.28*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.2 ± 0.07*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50 ± 0.17*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.1 ± 0.98*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3 Alcoholic extract</td>
<td>4.70 ± 0.1*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.10 ± 0.55</td>
<td>3.15 ± 0.11*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.2 ± 1.86</td>
<td>0.81 ± 0.17*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.8 ± 0.25</td>
</tr>
<tr>
<td>G4 Aqueous extract</td>
<td>4.30 ± 0.2*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.30 ± 0.31</td>
<td>3.21 ± 0.19*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.80 ± 1.13</td>
<td>0.93 ± 0.21*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.9 ± 0.97</td>
</tr>
</tbody>
</table>

Values are expressed as mg/24 h

*Newman keul’s multiple range test was used (*P<0.05)
*a different from G1  *b different from G2

### Table-4.4 Inhibitory effect DML on ethylene glycol induced hyperoxaluria

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Vol of urine ml/24 h</th>
<th>Calcium mg/24 h</th>
<th>Oxalate mg/24 h</th>
<th>Phosphate mg/24 h</th>
<th>Magnesium mg/24 h</th>
<th>Protein mg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Control</td>
<td>5.30 ± 0.2</td>
<td>0.59 ± 0.29</td>
<td>0.38 ± 0.02</td>
<td>5.90 ± 0.52</td>
<td>0.89 ± 0.06</td>
<td>2.78 ± 0.39</td>
</tr>
<tr>
<td>G2 Lithiatic control</td>
<td>2.90 ± 0.3*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.60 ± 0.34*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.20 ± 0.28*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.2 ± 0.07*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50 ± 0.17*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.1 ± 0.98*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3 Alcoholic extract</td>
<td>3.90 ± 0.1*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.40 ± 0.3</td>
<td>3.45 ± 0.01*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.8 ± 1.01</td>
<td>0.77 ± 0.21*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.2 ± 0.7</td>
</tr>
<tr>
<td>G4 Aqueous extract</td>
<td>4.10 ± 0.1*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.50 ± 0.19</td>
<td>3.50 ± 0.02*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.1 ± 1.04</td>
<td>0.80 ± 0.19*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.0 ± 0.6</td>
</tr>
</tbody>
</table>

Values are expressed as mg/24 h

Newman keul’s multiple range test was used (*P<0.05)
*a different from G1  *b different from G2
Effect of DRL on ethylene glycol induced hyperoxaluria

Fig. 4.3

Effect of DML on ethylene glycol induced hyperoxaluria

Fig. 4.4
4.4.2 Results

Ethylene glycol administration in G2 resulted in the elevation of the levels of calcium, oxalate, phosphate and proteins confirming the induction of lithiasis. The treatment with alcoholic and aqueous extracts of DRL could reduce considerably the oxalate level only. However, this treatment elevated the magnesium level and the effect on calcium and oxalate levels was more pronounced with the alcoholic extract. The aqueous extract was more effective for magnesium and protein levels in bringing them towards normal. Likewise, alcoholic and aqueous extracts of DML reduced the urinary risk parameters to some extent and elevated the urinary magnesium. The DRL extracts were found to be more effective than the DML extracts.

4.4.3 Discussion

Ethylene glycol gets oxidized to oxalic acid resulting in hyperoxaluria which is a significant risk factor in the pathogenesis of renal stones\(^{35}\). This factor along with increased excretion of calcium results in the formation of calcium oxalate renal stones\(^{36}\). The extracts reduced the supersaturation of calcium and oxalate in urine, thereby reducing the risk tendency of stone formation. Increased excretion of phosphates along with oxalates results in calcium phosphate crystals which induce further deposition of calcium oxalate\(^{37}\). Protein excretion is reported to be high in hyperoxaluric rats as well as in stone formers. Urinary magnesium is reported to be an inhibitor of urolithiasis as low levels of magnesium are encountered in stone formers\(^{39}\). Similarly, increased levels of protein are also observed in stone formers. From these facts, it is clear that ethylene glycol administration induces lithiasis by elevating the levels of calcium, oxalate and phosphate and by lowering...
magnesium. Following treatment with the extracts of DRL and DML, the risk factors namely, oxalate and magnesium were altered and hence it is concluded that the test extracts are effective against ethylene glycol induced hyperoxaluria. Amongst the two extracts, the DRL extracts exhibited a better reduction of hyperoxaluria than the extracts of DML.

4.5 Antifibrotic activity

Liver fibrosis results from repeated incidents of chronic liver damage\(^40\). During chronic liver damage, the extra-cellular protein matrix seems to be altered and the collagen content was found to be high which is stabilized by the triple helix structure of hydroxyproline (HP). Hence estimation of HP is an excellent marker for liver fibrosis due to repeated liver damage. Liver damage can be induced by bile duct ligation\(^41\) or by CC1\(_4\) administration\(^42\). In the present study, the protective effect of extracts of roots of \(D. rubiginosa\) (DRR) and \(D. malabarica\) (DMR) against CC1\(_4\), induced liver fibrosis has been studied.

4.5.1 Methodology

Liver fibrosis was induced following the method of Nan et al.\(^43\). The animals were divided into two batches, each consisting of four groups of six animals each.

4.5.1.1 Induction of liver fibrosis

CC\(_1\)\(_4\) was given to rats orally (1 ml/100g) mixed with an equal volume of liquid paraffin twice a week for 28 days. The extracts were suspended in 1% CMC and given orally by gavage for 28 days at a daily dose of 100 mg/kg. The rats were sacrificed under ether anesthesia,
three days after treatment with the last dose and blood and liver samples were collected. The treatment protocol followed is as follows:

In both the batches G1 was reserved as normal negative control; G2 as positive fibrotic control and G3 & G4 as groups treated with alcoholic and aqueous extracts of DRR and DMR respectively.

4.5.1.2 Determination of serum biochemical parameters

After 28 days of treatment, the rats were anaesthetized with ether and blood was collected through cardiac puncture for serum biochemical analysis. Blood samples were kept at room temperature for one hour and centrifuged at 3000 rpm for 3 min. to obtain the serum. The levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TBL) were estimated using auto analyzer.

4.5.1.3 Estimation of hydroxy proline

Hydroxyproline (HP) is a basic amino acid found in high percentage in basic proteins. There is a several fold increase in HP content under physiological and pathological stress conditions. HP content was estimated following the method of Jamal et al. The results are shown in Table-4.5 & Table-4.6 and presented in Fig. 4.5, 4.5.1 & Fig. 4.6, 4.6.1 for DRR and DMR respectively.
Table 4.5 Effect of DRR on biochemical parameters altered by CCI administration

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>AST U/l</th>
<th>ALT U/l</th>
<th>ALP U/l</th>
<th>TBL mg%</th>
<th>HP (g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>169.6 ± 1.80</td>
<td>56.0 ± 0.63</td>
<td>216.4 ± 2.9</td>
<td>0.50 ± 0.02</td>
<td>50.0 ± 0.3</td>
</tr>
<tr>
<td>G2 Fibrotic control</td>
<td>255.3 ± 6.22*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.15 ± 1.48*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>442.5 ± 2.32*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.21 ± 0.83*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.0 ± j</td>
</tr>
<tr>
<td>G3 Alcoholic extract</td>
<td>180.6 ± 5.41*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.48 ± 2.21*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>307.0 ± 3.1*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.55 ± 0.21*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.0 ± 2.1*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4 Aqueous extract</td>
<td>173.4 ± 4.1*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.61 ± 2.5*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>321.5 ± 4.1*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.56 ± 0.5*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>103.1 ± 3.1*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

*<sup>a</sup> different from G1  *<sup>b</sup> different from G2

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Table 4.6 Effect of DMR on biochemical parameters altered by CCI administration

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>AST U/l</th>
<th>ALT U/l</th>
<th>ALP U/l</th>
<th>TBL mg%</th>
<th>HP (g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>169.6 ± 1.80</td>
<td>56.0 ± 0.63</td>
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</tr>
<tr>
<td>G2 Fibrotic control</td>
<td>255.3 ± 6.22*&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>442.5 ± 2.32*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.21 ± 0.83*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.0 ± 1.7*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3 Alcoholic extract</td>
<td>165 ± 4.3*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.1 ± 1.9*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>286 ± 7.6*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53 ± 0.1*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.0 ± 3.4*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4 Aqueous extract</td>
<td>159 ± 3</td>
<td>66.0 ± 2.1*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>259 ± 6.9*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.56 ± 0.5*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.0 ± 4.7*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

*<sup>a</sup> different from G1  *<sup>b</sup> different from G2
Effect of DRR on biochemical parameters altered by CCl₄

Fig. 4.5

Fig. 4.5.1
Effect of DMR on biochemical parameters altered by CCl₄

Fig. 4.6

Fig. 4.6.1
4.5.2 Results

The investigation of biochemical parameters revealed that all the parameters viz. AST, ALT, ALP, TBL and HP were increased following CC1\textsubscript{4} treatment, as evident from the values of G2. The increased parameters observed in G2 were reduced by treatment with alcoholic and aqueous extracts of DRR (100 mg/kg). The results of G3 showed that AST, ALT, ALP, TBL and HP were brought to normal or near normal levels. The statistically significant increase in biochemical parameters observed in G2 indicated the hepatic damage induced by CC1\textsubscript{4}. Following treatment with the test extracts, the increase in all the parameters were considerably reduced. This suggested the extracts to have statistically significant antifibrotic activity. Similar results, observed with the extracts of DMR were found to be more effective than the extracts of DRR.

4.5.3 Discussion

Trichloromethyl radical is a bioactive species formed from CC1\textsubscript{4} by the liver cytochrome P\textsubscript{450}. This free radical initiates lipid peroxidation resulting in liver damage leading to liver fibrosis. As liver is damaged, the transaminases are elevated and also alkaline phosphatase (ALP) and total bilirubin (TBL). These biochemical markers suggested the damage of the liver. As the extent of liver fibrosis could be assessed by the HP content which gets elevated during chronic liver damage for evaluation of the antifibrotic effect of any agent, HP can serve as a biochemical marker\textsuperscript{45}. In our study too, HP content was found to be profoundly high in G2 (positive control) suggestive of liver fibrosis. As the extracts of DRR and DMR reduced the HP content, it is concluded that they are effective against CC1\textsubscript{4}, induced liver fibrosis. The DMR extracts are found to be more effective than the DRR extract against CC1\textsubscript{4}, induced liver fibrosis.
4.6 Hepatoprotective activity

Most of the enzymes of our body are synthesized in liver. Liver participates in a variety of functions ranging from synthesis of bile to detoxification mechanisms. The functional integrity of liver can be assessed by the enzyme levels, by measuring the bile flow and bile pigments. A number of hepato-toxins damage the liver and its functional capacity. A single dose of CC1\textsubscript{4} (0.75 ml/kg), a well known hepato-toxin is sufficient to induce liver damage indicated by abnormally high levels of enzymes like transaminases. The hepato protective activity of the extracts of leaves of *D. rubiginosa* (DRL) and *D. malabarica* (DML) were evaluated in albino rats.

4.6.1 Methodology

The method of Anand *et al.*\textsuperscript{46} was followed for evaluation of hepato protective activity of *D. malabarica* and *D. rubiginosa*. The animals were divided into two batches, each consisting of four groups of six animals each. The treatment protocol given is as follows:

In both the batches, G1 was kept as normal control, G2 as CC1\textsubscript{4} control, G3 and G4 as groups treated orally with alcoholic and aqueous extracts of DRL and DML respectively at a dose level of 100 mg/kg. CC1\textsubscript{4} (0.75 ml/kg) mixed with an equal volume of liquid paraffin was administered orally by gastric intubation. The animals of G3 and G4 were treated with 100 mg/kg of alcoholic and aqueous extracts of DRL and DML respectively 2 hours before CCI\textsubscript{4} intoxication. Blood was collected from animals of all the groups 18 hours after CCI\textsubscript{4} administration and serum was estimated for AST\textsuperscript{47}, ALT\textsuperscript{47}, ALP\textsuperscript{48}, serum bilirubin\textsuperscript{49} and total proteins\textsuperscript{34}. The results are tabulated in Table-4.7 & Table-4.8 and
presented in Fig. 4.7, 4.7.1 & Fig. 4.8, 4.8.1 for DRL and DML respectively.

Table 4.7 Effect of DRL on CCl₄ induced hepato-toxicity in rats

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>AST U/l</th>
<th>ALT U/l</th>
<th>ALP U/l</th>
<th>Total Bilirubin mg%</th>
<th>Total Protein g%</th>
<th>Liver weight g</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Control</td>
<td>138.2 ± 2.1</td>
<td>47.2 ± 1.8</td>
<td>180.7 ± 5.2</td>
<td>0.69 ± 0.02</td>
<td>9.43 ± 0.31</td>
<td>3.80 ± 0.08</td>
</tr>
<tr>
<td>G2 Positive control</td>
<td>279.8 ± 1.8*</td>
<td>101.2 ± 2</td>
<td>395.6 ± 8.6*</td>
<td>1.30 ± 0.03*</td>
<td>9.17 ± 0.28</td>
<td>7.10 ± 0.33***</td>
</tr>
<tr>
<td>G3 Alcoholic extract</td>
<td>161.2 ± 1.9*b</td>
<td>55.6 ± 1.9*b</td>
<td>280.4 ± 5.6*b</td>
<td>0.91 ± 0.05*b</td>
<td>9.44 ± 0.29</td>
<td>5.10 ± 0.29*b</td>
</tr>
<tr>
<td>G4 Aqueous extract</td>
<td>159.3 ± 1.6*b</td>
<td>61.3 ± 1.9*b</td>
<td>255.0 ± 6.1*b</td>
<td>0.88 ± 0.03*b</td>
<td>9.32 ± 0.25</td>
<td>4.90 ± 0.27*b</td>
</tr>
</tbody>
</table>

Newman’s multiple range test was used (*P<0.05)
*a different from G1 *b different from G2

Table 4.8 Effect of DML on CCl₄ induced hepato-toxicity in rats

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>AST U/l</th>
<th>ALT U/l</th>
<th>ALP U/l</th>
<th>Total Bilirubin mg%</th>
<th>Total Protein g%</th>
<th>Liver weight g</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Control</td>
<td>138.2 ± 2.1</td>
<td>47.2 ± 1.8</td>
<td>180.7 ± 5.2</td>
<td>0.69 ± 0.02</td>
<td>9.43 ± 0.31</td>
<td>3.80 ± 0.08</td>
</tr>
<tr>
<td>G2 Positive control</td>
<td>279.8 ± 1.8*a</td>
<td>101.2 ± 2.9*a</td>
<td>395.6 ± 8.6*</td>
<td>1.30 ± 0.03*a</td>
<td>9.17 ± 0.28</td>
<td>7.10 ± 0.33*a</td>
</tr>
<tr>
<td>G3 Alcoholic extract</td>
<td>179.0 ± 7.6*b</td>
<td>71.0 ± 4.5*b</td>
<td>285.0 ± 6.6*b</td>
<td>0.93 ± 0.1*b</td>
<td>9.51 ± 0.31</td>
<td>5.30 ± 0.2*b</td>
</tr>
<tr>
<td>G4 Aqueous extract</td>
<td>173.0 ± 6.6*b</td>
<td>68.0 ± 3.9*b</td>
<td>283.0 ± 4.5*b</td>
<td>0.92 ± 0.2*b</td>
<td>9.49 ± 0.41</td>
<td>5.50 ± 0.2*b</td>
</tr>
</tbody>
</table>

Newman’s multiple range test was used (*P<0.05)
*a different from G1 *b different from G2
Effect of DRL on \( \text{CCl}_4 \) induced hepatotoxicity

Fig. 4.7

![Graph showing AST, ALT, and ALP levels](image)

Fig. 4.7.1

![Graph showing total bilirubin, total protein, and liver weight](image)
Effect of DML on CCl₄ induced hepatotoxicity

Fig. 4.8

Fig. 4.8.1
4.6.2 Results

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 by liver and concentrated in gall bladder. Estimation of bile pigments adds support to the extent of liver injury. In our study, following CC1 administration, there was a significant increase in total bilirubin which was reduced to near normal values after administration of DRL extracts. However, protein content was not altered by CC1 treatment. The extracts of DML showed a similar pattern of results, which are less effective when compared to DRL.

4.6.3 Discussion

Trichloromethyl, a free radical formed from CC1, 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. Both the test extracts maintained the integrity of liver cell membrane as evidenced by significant reduction of AST and ALT levels hiked by CC1, administration. Reduction in serum bilirubin indicated the restoration of the functional status of liver. The increase in liver weight after CC1, treatment is due to its enlargement. The extracts were able to reduce the enlarged liver showing significant hepatoprotective activity for the extracts of D. rubiginosa and D. malabarica.
4.7 Antidiabetic activity

Diabetes mellitus is a metabolic disorder that deranges carbohydrate, lipid and protein metabolism. It is characterized by hyperglycemia and glycosuria and is associated with insulin deficiency. In experimental animals, various agents like alloxan, diazoxide and streptazotocin are used to induce diabetes. 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 extracts of roots of *D. rubiginosa* (DRR) and *D. malabarica* (DMR).

4.7.1 Methodology

The antidiabetic activity was evaluated by the method followed by Kameswara Rao *et al.* 51. 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% fasting blood glucose were selected and were divided into two batches, each consisting of five groups of six animals each. The treatment protocol is as follows:

In each batch, G1 was reserved as diabetic control and G2 was treated with tolbutamide (100 mg/kg) p.o. as standard drug, G3 and G4 were given alcoholic and aqueous extracts of DRR and DMR, each at a dose of 100 mg/kg p.o. respectively.

Blood samples, collected from the individual groups of animals at two different intervals of time (5th & 10th hour) after treatment with the extracts and tolbutamide were analyzed for blood glucose. The results are
tabulated in Table-4.9 and Table-4.10 and presented in Fig. 4.9 & Fig. 4.10 for DRR and DMR respectively.

Table-4.9 Effect of DRR 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>Oh</td>
</tr>
<tr>
<td>G1 Diabetic control</td>
<td>308.0 ± 1.3</td>
</tr>
<tr>
<td>G2 Standard</td>
<td>325.0 ± 2.2</td>
</tr>
<tr>
<td>G3 Alcoholic extract</td>
<td>318.0 ± 3.4</td>
</tr>
<tr>
<td>G4 Aqueous extract</td>
<td>321.0 + 4.1</td>
</tr>
</tbody>
</table>

Newman keul’s multiple range test was used
* significantly different from zero hour reading (*P<0.05)

Table-4.10 Effect of DMR 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>G1 Diabetic Control</td>
<td>308.0 ± 1.3</td>
</tr>
<tr>
<td>G2 Standard</td>
<td>325.0 ± 2.2</td>
</tr>
<tr>
<td>G3 Alcoholic Extract</td>
<td>318.0 ± 3.1</td>
</tr>
<tr>
<td>G4 Aqueous Extract</td>
<td>320.0 + 2.1</td>
</tr>
</tbody>
</table>

Newman keul’s multiple range test was used
* significantly different from zero hour reading (*P<0.05)
Effect of DRR on blood glucose levels of alloxan induced diabetic rats

Fig. 4.9

Effect of DMR on blood glucose levels of alloxan induced diabetic rats

Fig. 4.10
4.7.2 Results

It is evident that the alcoholic and aqueous extracts were able to lower the elevated blood glucose level at the 5th & 10th hour of administration. The reduction was more effective at the 5th hour than the 10th hour for DRR extract and vice-versa for DMR extract. Among the extracts used, the effect on the hyperglycemic rats was found to be more pronounced for the aqueous extract. However, the hypoglycemic effect for the extracts was found to be less when compared with that of the standard drug tolbutamide.

4.7.3 Discussion

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. The extracts when evaluated after one week of administration of alloxan, showed moderate antidiabetic effect on prolonged hyperglycemic phase.

4.8 Diuretic activity

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, various extracts of roots of *D. rubiginosa* (DRR) and *D. malabarica* (DMR) have been screened for their diuretic activity.
4.8.1 Methodology

The method of Lipschitz was followed to evaluate the diuretic activity. Male albino Wistar rats, each weighing 150-200 g selected for the study were divided into two batches, each consisting of six groups of six animals each. The treatment protocol followed is as follows:

G1 was kept as normal control and G2 was given the standard drug frusemide. G3, G4, and G5 received chloroform, alcoholic and aqueous extracts of DRR and DMR at a dose of 100 mg/kg 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 chloroform, alcoholic and aqueous extracts at a dose of 100 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 were estimated by flame photometry and chloride and bicarbonate ions by titrimetry. The results are shown in Table-4.11 & Table-4.12 and Fig. 4.11.1 & Fig. 4.12, 4.12.1 for DRR and DMR respectively.
Table-4.11  Diuretic potential of DRR

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Volume of urine (ml)</th>
<th>Na⁺ (meq/L)</th>
<th>Cl⁻ (mmol/L)</th>
<th>HCO₃⁻ (mmol/L)</th>
<th>K⁺ (meq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Control</td>
<td>3.10 ± 2.4</td>
<td>64.0 ± 1.47</td>
<td>84.0 ± 1.47</td>
<td>12.2 ± 1.11</td>
<td>14.48 ± 3.71</td>
</tr>
<tr>
<td>G2 Standard</td>
<td>6.73 ± 3.41**a</td>
<td>440.0 ± 2.81*a</td>
<td>145.0 ± 3.01*a</td>
<td>24.3 ± 2.11*a</td>
<td>15.20 ± 4.11*a</td>
</tr>
<tr>
<td>G3 Chloroform</td>
<td>3.90 ± 4.31*a</td>
<td>80.0 ± 3.5*a</td>
<td>89.0 ± 2.11*a</td>
<td>13.0 ± 5.1*b</td>
<td>14.73 ± 5.1*b</td>
</tr>
<tr>
<td>G4 Alcoholic</td>
<td>8.50 ± 1.17*a</td>
<td>368.0 ± 3.11*a</td>
<td>132.9 ± 2.01*a</td>
<td>20.0 ± 1.11*a</td>
<td>16.0 ± 3.10*a</td>
</tr>
<tr>
<td>G5 Aqueous</td>
<td>7.90 ± 2.31*a</td>
<td>24.0 ± 3.5*a</td>
<td>140.0 ± 1.11*a</td>
<td>21.0 ± 2.11*a</td>
<td>15.91 ± 2.11*a</td>
</tr>
</tbody>
</table>

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

*a – different from G1    *b – different from G2    n.s– non-significant

Table-4.12  Diuretic potential of DMR

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Volume of urine (ml)</th>
<th>Na⁺ (meq/L)</th>
<th>Cl⁻ (mmol/L)</th>
<th>HCO₃⁻ (mmol/L)</th>
<th>K⁺ (meq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Control</td>
<td>3.10 ± 0.47</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>G2 Standard</td>
<td>6.73 ± 3.41*a</td>
<td>440.0 ± 2.81*a</td>
<td>145.0 ± 3.01*a</td>
<td>24.3 ± 2.11*a</td>
<td>15.20 ± 4.11*a</td>
</tr>
<tr>
<td>G3 Chloroform</td>
<td>3.50 ± 2.71*a</td>
<td>120.0 ± 5.21*a</td>
<td>80.1 ± 3.11*a</td>
<td>10.0 ± 4.8*a</td>
<td>14.6 ± 4.8*a</td>
</tr>
<tr>
<td>G4 Alcoholic</td>
<td>7.60 ± 3.69*b</td>
<td>190.0 ± 1.2*b</td>
<td>146.0 ± 1.2*b</td>
<td>19.7 ± 1.2*b</td>
<td>15.1 ± 1.2*b</td>
</tr>
<tr>
<td>G5 Aqueous</td>
<td>8.10 ± 4.7*b</td>
<td>204.0 ± 7.4*b</td>
<td>151.0 ± 3.1*b</td>
<td>18.9 ± 3.1*b</td>
<td>15.8 ± 3.1*b</td>
</tr>
</tbody>
</table>

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

*a – different from G1    *b – different from G2    n.s– non-significant
Diuretic potential of DRR

Fig. 4.11

![Graph showing Na⁺ (meq/L) and Cl⁻ (mmol/L) for different groups (G1 to G5).]

Fig. 4.11.1

![Graph showing Vol. of urine (ml/24 h), HCO₃⁻ (mmol/L), and K⁺ (meq/L) for different groups (G1 to G5).]
Diuretic potential of DMR
Fig. 4.12

Fig. 4.12.1
4.8.2 Results

The alcoholic extract of DRR significantly increased the volume of urine followed by the aqueous and chloroform extracts. Likewise the alcoholic extract showed increased excretion of sodium ions followed by chloroform and aqueous extracts. Excretion of chloride and bicarbonate ions was high with the aqueous extract and it was reduced considerably by alcoholic and chloroform extracts.

In case of DMR, the volume of urine and excretion of sodium, potassium and chloride ions are significantly increased by the aqueous, chloroform and alcoholic extracts and excretion of bicarbonate ions was observed to be high for chloroform extract, followed by aqueous and alcoholic extracts.

4.8.3 Discussion

The alcoholic extract of both DRR and DMR enhances the urine volume considerably, which is more than that with frusemide. The increase in sodium and potassium ion excretion showed that the test extracts produce natriuretic and kaliuretic effects. In our study, the chloride ion excretion is also observed to be high. Though the standard drug employed is a high ceiling diuretic, the extracts are found to induce diuresis comparable to that of the standard. The DRR extracts showed better effects on the volume of urine output, bicarbonate and potassium excretion and the DMR extracts exhibited more pronounced natriuresis and chloride ion excretion.
4.9 Anti-inflammatory activity

Inflammation is characterized by pain, redness, swelling and immobility. Inflammation occurs in three phases viz. acute inflammation, immune response and chronic inflammation\textsuperscript{55}. Many drugs of plant origin are reported to possess anti-inflammatory activity and most of them reduce the severity of the symptoms. Edema (accumulation of fluid in the tissues) is also a known symptom of inflammation. The anti-inflammatory drugs are assessed by their potential to reduce the experimentally induced edema. In the present study, the effect of extracts of leaves of \textit{D. malabarica} (DML) against carrageenin induced paw edema was assessed.

4.9.1 Methodology

The method of Winter \textit{et al.}\textsuperscript{56} was followed to evaluate the anti-inflammatory potential. Male Wistar rats were divided into five groups, each consisting of six animals. The treatment protocol followed is as follows:

G1 was kept as inflammatory control and G2 was given indomethacin as the standard drug (20 mg/kg s.c.). G3, G4 & G5 were treated with chloroform, alcoholic and aqueous extracts of DML at a dose of 100 mg/kg p.o. respectively.

Edema was induced by sub-plantar injection of 0.1 ml of 1% freshly prepared suspension of carrageenin into the right hind paw of each rat. The paw volume was measured by using a plethysmograph before administration of carrageenin, considered as 0 hour reading and also every hour after the injection of carrageenin. The extracts were
administered orally to the animals, an hour before the injection of carrageenin. The paw volume was measured at 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} hour after the administration of carrageenin. The results are tabulated in Table-4.13 and presented in Fig. 4.13.

Table-4.13 Effect of DML on carrageenin induced paw edema

<table>
<thead>
<tr>
<th>Group/ Treatment</th>
<th>% increase in paw volume after 3 h</th>
<th>% inhibition of increase in paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Positive control</td>
<td>31.3 ± 1.09</td>
<td>-</td>
</tr>
<tr>
<td>G2 Standard</td>
<td>16.4 ± 0.99*</td>
<td>47.6</td>
</tr>
<tr>
<td>G3 Chloroform extract</td>
<td>22.0 ± 0.76*</td>
<td>29.7</td>
</tr>
<tr>
<td>G4 Alcoholic extract</td>
<td>21.7 ± 1.21*</td>
<td>30.6</td>
</tr>
<tr>
<td>G5 Aqueous extract</td>
<td>23.1 ± 1.74*</td>
<td>26.1</td>
</tr>
</tbody>
</table>

Newman keul’s multiple range test was used
^significant against the control (*P<0.05)

4.9.2 Results

Sub-plantar injection of carrageenin resulted in an increase of paw volume (edema) that was maximum after 3 hours. Among the three groups, the alcoholic extract treated group showed a minimum increase in the paw volume after 3 hours and with the other two extracts, an increase in paw volume was observed. The percentage decrease in paw volume was calculated using the following formula:

\[
\text{% decrease in paw volume} = \left(\frac{C-T}{C}\right) \times 100
\]
Effect of DML on carrageenin induced paw edema

Fig. 4.13
where C and T are the paw volumes of inflammatory control group and the test extracts treated group. While maximum percentage decrease of 30.6% in paw volume was shown by the alcoholic extract, the other two extracts showed 29.7 and 26.1 % decrease in paw volume respectively. As the percentage decrease in paw volume for the standard was 47.6, it is inferred that none of the extracts was found to possess anti-inflammatory effect comparable to that of the standard drug.

The extracts of DRL and DML showed comparable results in most of the other biological activities taken up for our study. However in the case of anti-inflammatory screening, the results of DML were not encouraging, and hence the screening of DRL for its anti-inflammatory activity was not carried out.

4.9.3 Discussion

Carrageenin induces inflammation biphasically. The initial phase which lasts for an hour after administration of carrageenin is due to release of histamine and serotonin. The second phase is due to release of bradykinin and prostaglandins. The extracts showed a maximum reduction at third hour after administration of carrageenin and hence only these values are tabulated. From the data it is evident that the alcoholic extract showed a higher percentage decrease in paw volume. As this effect was observed at the third hour after carrageenin administration, it is clear that the drugs are effective in the second phase of carrageenin-induced inflammation.
4.10 Antipyretic activity

Regulation of body temperature is due to a balance between heat production and loss and hypothalamus is the centre for regulating body temperature. During fever, this balance is deranged and 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\(^{58}\).

4.10.1 Methodology

The antipyretic activity of the extracts of roots of *D. malabarica* (DMR) was studied against yeast-induced pyrexia by following the method of Smith and Hambourger\(^{59}\). Male Wistar rats were divided into five groups, each consisting of six animals. The treatment protocol followed is as follows:

G1 was kept as pyretic control, G2 received the standard drug aspirin, G3, G4 and G5 as groups treated with chloroform, alcoholic and aqueous extracts of DMR.

The basal temperature was recorded using a rectal tele thermometer 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. The extracts were given orally at a dose of 100 mg/kg p.o. after 10 hours of the injection of yeast and the standard drug aspirin was administered orally at a dose of 90 mg/kg. The body temperature was recorded after 5\(^{th}\) and 10\(^{th}\) hour. The results are tabulated in Table-4.14.
Table-414 Antipyretic effect of DRR

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Body temperature (°C)</th>
<th>Before yeast injection</th>
<th>After drug administration 5h</th>
<th>After drug administration 10h</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Pyretic control</td>
<td>37.6 ± 0.03</td>
<td>39.8 ± 0.03</td>
<td>39.7 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>G2 Standard</td>
<td>37.4 ± 0.04</td>
<td>37.6 ± 0.02*</td>
<td>37.5 ± 0.03*</td>
<td></td>
</tr>
<tr>
<td>G3 Chloroform extract</td>
<td>37.9 ± 0.02</td>
<td>38.1 ± 0.03*</td>
<td>38.4 ± 0.01*</td>
<td></td>
</tr>
<tr>
<td>G4 Alcoholic extract</td>
<td>37.2 ± 0.03</td>
<td>37.9 ± 0.04*</td>
<td>38.3 ± 0.02*</td>
<td></td>
</tr>
<tr>
<td>G5 Aqueous extract</td>
<td>37.2 ± 0.03</td>
<td>38.3 ± 0.04*</td>
<td>38.6 ± 0.03*</td>
<td></td>
</tr>
</tbody>
</table>

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

4.10.2 Results

It was found that all the extracts at a dose of 100 mg/kg, lower body temperature moderately after 5 hours of administration. Among the three extracts screened, the alcoholic extract showed a maximum decrease in body temperature and the other two showed a very minimum decrease only, not comparable with the standard drug.

4.10.3 Discussion

Antipyretics are agents that reduce the elevated body temperature. Analgesic and anti-inflammatory effects are normally associated with antipyretic activity. In addition to the antipyretic effect, most of the antipyretics possess analgesic and anti-inflammatory activities. The antipyretics usually reduce the body temperature by increasing the heat loss. In the present study, a very minimum reduction in the body
Analgesic activity of DMR by Hot Plate method
Fig. 4.14

[Bar chart showing basal reaction time after drug administration (in seconds) for different groups over time (0 min, 30 min, 60 min, 90 min, 120 min).]

Analgesic activity of DMR by Caudal Compression method
Fig. 4.15

[Bar chart showing basal reaction time after drug administration (in seconds) for different groups over time (0 min, 30 min, 60 min, 90 min, 120 min).]
temperature was observed with the extracts. Screening of the test extracts for their analgesic activity showed them to possess analgesia, though feeble, as compared with the standard drug aspirin. In our study, the extracts exhibited antipyretic effect in addition to the analgesic effect, as in the case of many of the established antipyretics.

4.11 Analgesic activity

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 method are used to evaluate the analgesic effect of the extracts of roots of *D. malabarica* (DMR). In both the analgesic methods, the latency between the application of obnoxious stimuli and the response was considered as the ‘basal reaction time’.

4.11.1 Methodology

4.11.1.1 Hot Plate method

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

G1 kept as normal control, received 2 ml of 1% CMC and G2 received the standard drug pentazocine at a dose of 60 mg/kg i.p. G3, G4 and G5 were administered with chloroform, alcoholic and aqueous extracts of DMR respectively, at a dose of 100 mg/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 upto 2 hours and the results are shown in Table-4.15 and presented in Fig. 4.14.

Table-4.15 Analgesic activity of DMR by Hot Plate method

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Basal reaction time after drug administration (sec) M+SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Gl Control</td>
<td>3.20 ± 0.25</td>
</tr>
<tr>
<td>G2 Standard</td>
<td>3.50 ± 0.15</td>
</tr>
<tr>
<td>G3 Chloroform extract</td>
<td>3.40 ± 0.1</td>
</tr>
<tr>
<td>G4 Alcoholic extract</td>
<td>3.25 ± 0.2</td>
</tr>
<tr>
<td>G5 Aqueous extract</td>
<td>3.60 ± 0.25</td>
</tr>
</tbody>
</table>

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

4.11.2 Results

The latency between the noxious stimulus and the response was prolonged by pentazocine and all the three extracts of our study after an hour of their administration. However the latency was maintained at normal control level in Gl.

4.11.1.2 Caudal compression method

The method of Bianchi et al. was followed. Swiss albino mice, each weighing 25-30 g, selected for the study were randomly divided into
five groups of six animals each. The treatment protocol followed is as follows: G1 received 2 ml of 1% CMC and kept as normal control and G2 received the standard drug pentazocine at a dose of 60 mg/kg i.p. G3, G4 and G5 were given chloroform, alcoholic and aqueous extracts of DMR at a dose of 100 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 the extracts. The latency was measured at an interval of 30 min. upto 2 hours. The results are shown in Table-4.16 and presented in Fig. 4.15.

Table-4.16 Analgesic activity of DMR by Caudal compression method

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Basal reaction time after drug administration (sec) M+SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>G1 Control</td>
<td>2.80 ±0.2</td>
</tr>
<tr>
<td>G2 Standard</td>
<td>3.50 ±0.23</td>
</tr>
<tr>
<td>G3 Chloroform extract</td>
<td>3.60 ±0.3</td>
</tr>
<tr>
<td>G4 Alcoholic Extract</td>
<td>3.40 ±0.2</td>
</tr>
<tr>
<td>G5 Aqueous extract</td>
<td>3.10 ±0.3</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.
4.11.3 Results

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 all the extracts at 30 min. after drug administration and the maximum prolongation of latency was seen after 120 min. for chloroform and alcoholic extracts and after 60 min. for the aqueous extract.

4.11.4 Discussion

Though many methods are available to evaluate analgesic activity, Eddy’s hot plate method is commonly used to evaluate centrally acting analgesics. The extracts were 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, the test extracts showed an effect lasting upto the 120th min. of study whereas with pentazocine, the effect started fading after 90th min. It is perhaps because the extracts act through peripheral mechanism as well.
References


