CHAPTER-5

Pharmacological Investigations of Crude Extracts

Section A:  Antifertility activity in female rats
Section B:  Antifertility activity in male rats
Section C:  Anthelmintic activity
Section D:  Anti-inflammatory activity
Section E:  Analgesic activity
Section F:  Anti-catatonic activity
Section G:  Diuretic activity
Section H:  Hepatoprotective activity
Section I:  Antioxidant activity


The root of *Momordica dioica* Roxb. is known to exhibit certain biological and pharmacological activities\(^1\)\(^-\)\(^3\). Hence, it was contemplated to subject the crude extracts of the root of this plant for investigation for the following activities.

1. Antifertility in female albino rats
2. Antifertility in male albino rats
3. Anthelmintic
4. Anti-inflammatory
5. Analgesic
6. Anticatatonic
7. Diuretic
8. Hepatoprotective
9. Antioxidant

The main aim of the present research work was to find out non-steroidal active constituents of the plant. Hence, only the ethanolic extract—both soluble and insoluble part and aqueous extract were taken up for the screening purpose, as the chloroform and petroleum ether extracts were shown to contain steroids. Emphasis is given to antifertility activity because of our interest in isolating suitable, non-steroidal emmenagogue of plant origin.
5.1 Antifertility activity study of crude extracts of the root of *Momordica dioica* Roxb.

Antifertility effect of any drug in female can be ascertained by studying the following parameters.

- Anti-implantation
- Abortifacient
- Estrogenic / antiestrogenic

5.1.1 Postcoital antifertility testing in female rats:

Post coital antifertility testing was carried out in female Wistar rats as per the method described by Khanna and Chaudhury\(^4\). Wistar strain female albino rats weighing between 152-200 g of proven fertility and with regular estrous cycle were selected for this experiment. These female rats were monitored daily for different stages of estrous cycle. The rats which were in proestrous stage were separated and mated with male rats, that had sired litters before, in the ratio of 2:1 in separate cages. Every morning the vaginal smears were examined for the presence of lumps of spermatozoa. The day when spermatozoa were detected in the vaginal smear was considered as day 1 of pregnancy. Then the pregnant rats were isolated. The extracts were administered at the dose of 200 mg/kg p.o. through intragastric catheter from day 1 to day 7 of the pregnancy. The animals were laprotomised under light ether anaesthesia on day 10 of pregnancy and both the horns of the uterus were observed for number, color and size of implants (Fig.5 and 6). Simultaneously, the number of corpora lutea were also noted. The abdomen was sutured and the pregnancy was allowed to go to full term. After the delivery, the litters were counted and examined for evidence of teratogenicity. The observation made are presented in Table-5.1.
Figure-5  Photograph of the uterus of a rat with ovary without implantation sites.

Figure-6  Photograph of the uterus of the control rat on day 10 of pregnancy showing the implantation sites.
Figure-5

Figure-6
Table-5.1: Abortifacient activity of the root of *Momordica dioica* Roxb.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>No. of rats</th>
<th>No. of rats with implantation on day 10</th>
<th>No. of implantation sites in individual rats on day 10</th>
<th>No. of rats delivered</th>
<th>Abortifacient activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td></td>
<td>6</td>
<td>6</td>
<td>10,11,9,9,9,10</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>II</td>
<td>Aqueous extract</td>
<td>200 mg/kg</td>
<td>6</td>
<td>6</td>
<td>9,11*,7,10,11,9</td>
<td>1</td>
<td>83.33</td>
</tr>
<tr>
<td>III</td>
<td>Ethanolic extract</td>
<td>(soluble part)</td>
<td>200 mg/kg</td>
<td>6</td>
<td>7,7,6,8,9,9</td>
<td>Nil</td>
<td>100</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanolic extract</td>
<td>(insoluble part)</td>
<td>200 mg/kg</td>
<td>6</td>
<td>8,9,7,10,9,6</td>
<td>Nil</td>
<td>100</td>
</tr>
</tbody>
</table>

* out of 11 implants, only 5 litters were born.

Encouraged by the findings of the antifertility testing, more detailed studies were carried out to investigate the possible mechanism of their antifertility action.

### 5.1.2 Estrogenic and antiestrogenic activity:

For studying estrogenic and antiestrogenic activity, young female rats (21-23 d) weighing between 35-40 g were bilaterally ovariectomised under light ether anaesthesia and sterile conditions. They were divided into 8 groups consisting of 6 rats each. The first group served as the control and received vehicle only (Tween-80, 2%, 5 ml/kg). The second group received ethinyl estradiol in olive oil (1 μg/rat/day) subcutaneously. The third, fourth and fifth groups correspondingly received aqueous extract, ethanolic extract-soluble part and ethanolic extract insoluble part at a dose of 200 mg/kg p.o. suspended in aqueous solution of Tween-80 (2%). The sixth, seventh and eighth groups received, in
addition to ethinyl estradiol, aqueous extract, ethanolic extract-soluble part and ethanol extract insoluble part at the dose of 200 mg/kg p.o. respectively. All the above treatments were given for 7 days. Vagina and vaginal smears were examined in all the animals in the treated groups for 7 days of treatment. On day 8, the rats were sacrificed by decapitation, the uteri dissected out, weighed and fixed in Bouin’s fluid for 25 h for histological studies. The diameter of the uterus, thickness of endometrium and the height of endometrial epithelium were measured in 10 randomly selected sections using a calibrated ocular micrometer [(Fig. 7-14 and 14(a)]. The statistical analysis was carried out by using student’s ‘t’ test. The results were expressed as mean ± sem. The findings are summarized in Tables-5.2 and 5.3.
Figure-7 Photograph showing the cross section of the uterus of immature control rat showing normal myometrium and very few endometrial glands x 100.

Figure-8 Photograph of the cross section of the uterus of immature rat treated with ethinyl estradiol at 1 μg/rat/day showing increase in the height of the myometrium, endometrium and endometrial glands x 250.

ED    Endometrium
EG    Endometrial gland
EP    Epithelium
MY    Myometrium
L     Lumen
Figure-9  Photograph showing the cross section of the uterus of immature rat treated with aqueous extract of the root of *Momordica dioica* Roxb. at 200 mg/kg body weight showing mild estrogenic activity. Endometrial glands are more visible. Height of myometrium and endometrium is increased x 100.

Figure-10  Photograph showing cross section of the uterus of immature rat treated with aqueous extract of the root of *Momordica dioica* Roxb at 200 mg/kg body weight along with ethinyl estradiol at 1μg/rat/day showing the endometrial glands, reduction in lumen and increase in the thickness of endometrium x 100

ED  Endometrium
EG  Endometrial gland
EP  Epithelium
MY  Myometrium
L  Lumen
Figure-11 Photograph of the cross section of the uterus of the immature rat treated with ethanolic extract-soluble part of the root of *Momordica dioica* Roxb. at 200 mg/kg body weight showing estrogenic activity. There is marked increase in the height of the endometrial epithelium, thickness of endometrium. Endometrial glands are clearly seen x 250.

Figure-12 Photograph of the cross section of the uterus of the immature rat treated with ethanolic extract-soluble part of the root of *Momordica dioica* Roxb. at 200 mg/kg body weight along with ethinyl estradiol at 1μg/rat/day. The figure indicates significant estrogenic activity which could be seen by the increase in the height of endometrial epithelium, reduction in lumen, increase in the thickness of endometrium and appearance of endometrial glands x 250.

ED  Endometrium  
EG  Endometrial gland  
EP  Epithelium  
MY  Myometrium  
L   Lumen
Figure-13 Photograph of the cross section of the uterus of the immature rat treated with ethanolic extract-insoluble part of the root of *Momordica dioica* Roxb. at 200 mg/kg body weight. Mild estrogenic activity is seen. There is slight increase in the height of endometrial epithelium. Less number of endometrial glands are seen x 250.

Figure-14 Photograph of the cross section of the uterus of the immature rat treated with ethanolic extract-insoluble part of the root of *Momordica dioica* Roxb. at 200 mg/kg body weight along with ethinyl estradiol 1μg/rat/day. Estrogenic activity of the ethinyl estradiol is potentiated which could be noticed by the significant increase in the height of the endometrial epithelium, thickness of endometrium and increase in the diameter of the uterus.

<table>
<thead>
<tr>
<th>ED</th>
<th>Endometrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG</td>
<td>Endometrial gland</td>
</tr>
<tr>
<td>EP</td>
<td>Epithelium</td>
</tr>
<tr>
<td>MY</td>
<td>Myometrium</td>
</tr>
<tr>
<td>L</td>
<td>Lumen</td>
</tr>
</tbody>
</table>
Figure 14(a) shows the uterine weight (mg/100 g body weight) for different treatments. The chart compares the effects of Control, Aqueous extract, Ethanol extract-soluble part, Ethanol extract-insoluble part, and Ethanol extract-soluble part + Ethynyl estradiol on uterine weight. The extract (200 mg/kg) has a significant impact on the uterine weight compared to the control, with values ranging from 79 to 218 mg/100 g body weight.
Table 5.2: Estrogenic and antiestrogenic activity of various extracts

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (Dose)</th>
<th>Uterine weight mg/100g body wt. Mean ± SEM</th>
<th>Vaginal Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>78.50±2.72</td>
<td>---</td>
</tr>
<tr>
<td>II</td>
<td>Ethinyl estradiol (1 μg/rat/day)</td>
<td>179.12±2.15</td>
<td>nucleated and cornified cells</td>
</tr>
<tr>
<td>III</td>
<td>Aqueous extract(200 mg/kg)</td>
<td>104.83±2.09*</td>
<td>cornified cells</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanolic extract–soluble part (200 mg/kg)</td>
<td>111.85±3.48*</td>
<td>cornified cells</td>
</tr>
<tr>
<td>V</td>
<td>Ethanolic extract–insoluble part (200 mg/kg)</td>
<td>95.66±2.11</td>
<td>cornified cells</td>
</tr>
<tr>
<td>VI</td>
<td>Ethinyl estradiol (1 μg/rat/day) + Aqueous extract (200 mg/kg)</td>
<td>217.33±2.99*</td>
<td>nucleated and cornified cells</td>
</tr>
<tr>
<td>VII</td>
<td>Ethinyl estradiol (1 μg/rat/day) + Ethanolic extract–soluble part (200 mg/kg)</td>
<td>218.35±6.55*</td>
<td>nucleated and cornified cells</td>
</tr>
<tr>
<td>VIII</td>
<td>Ethanolic extract–insoluble part (200 mg/kg) and Ethinyl estradiol–1 μg/rat/day</td>
<td>182.66±5.75*</td>
<td>nucleated and cornified cells</td>
</tr>
</tbody>
</table>

*p <0.001 vs control, student’s 't'-test, n=6
Table-5.3: Histological changes in the uterus and endometrium observed for various treatments

<table>
<thead>
<tr>
<th>Treatment Dose</th>
<th>Height of endometrial epithelium (μm)±SEM</th>
<th>Thickness of endometrium (μm)±SEM</th>
<th>Diameter of uterus (μm)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.90±1.83</td>
<td>61.70±2.24</td>
<td>329.46±07.08</td>
</tr>
<tr>
<td>Ethinyl estradiol (1 μg/rat/day)</td>
<td>50.58±2.67</td>
<td>351.14±6.62</td>
<td>745.62±10.01</td>
</tr>
<tr>
<td>Aqueous extract (200 mg/kg)</td>
<td>39.02±1.94</td>
<td>121.38±7.11*</td>
<td>492.72±14.91*</td>
</tr>
<tr>
<td>Ethanolic extract–soluble part (200 mg/kg)</td>
<td>44.80±1.45</td>
<td>86.70±4.32*</td>
<td>445.03±08.27*</td>
</tr>
<tr>
<td>Ethanolic extract–insoluble part (200 mg/kg)</td>
<td>31.79±2.20</td>
<td>144.50±7.00*</td>
<td>533.70±14.40*</td>
</tr>
<tr>
<td>Ethinyl estradiol (1 μg/rat/day)+aqueous extract (200 mg/kg)</td>
<td>52.02±2.25*</td>
<td>484.05±21.68*</td>
<td>1030.30±16.99*</td>
</tr>
<tr>
<td>Ethinyl estradiol (1 μg/rat/day)+ethanolic extract–soluble part (200 mg/kg)</td>
<td>58.96±1.07</td>
<td>420.50±3.71*</td>
<td>926.20±06.86*</td>
</tr>
<tr>
<td>Ethinyl estradiol (1 μg/rat/day)+ethanolic extract–insoluble part (200 mg/kg)</td>
<td>44.80±2.41</td>
<td>368.50±16.86*</td>
<td>835.20±15.93*</td>
</tr>
</tbody>
</table>

* p <0.001 vs control, student’s 't'-test, n=6

5.1.3 Results:

All the extracts were evaluated for postcoital antifertility activity at the dose of 200 mg/kg p.o. The number of implantation sites were matching with the number of corpora lutea observed in each animal. There was no noticeable change in the color and size of the implants when compared with animals of control group. These observations
clearly indicate that none of the extracts possess anti-implantation activity. However, all the extracts exhibited significant abortifacient activity. Aqueous extract showed 83% abortifacient activity and ethanolic extract soluble and insoluble part exhibited 100% abortifacient activity. No vaginal bleeding was observed in the treated animals and no toxic effects were observed by gross visual examination. The action of the extracts were found to be reversible, as all the animals in the treated group returned back to regular estrus cycle and became pregnant to deliver normal litters.

The estrogenic and antiestrogenic activity of the extracts is shown in Tables-5.2 and 5.3. Oral administration of these extracts caused significant increase in uterine weight. The uterotrophic potency, as shown by the increase in weight of the uterus, was about 59% of that of ethinyl estradiol in the case of aqueous extract, 63% in the case of ethanolic extract-soluble part and 54% in the case of ethanolic extract-insoluble part, when compared with that of ethinyl estradiol, which showed mild estrogenic activity of these extracts. This fact was well supported by other parameters such as increase in diameter of the uterus, thickness of endometrium and height of endometrial epithelium.

It was observed that aqueous extract, ethanolic extract-soluble part and insoluble part possess 35%, 25% and 41% of estrogenic efficacy respectively, when compared to that of ethinyl estradiol with respect to the increase in the thickness of endometrium. It was also observed that the extracts potentiated the action of ethinyl estradiol when administered together. This fact was well indicated by the significant increase in diameter of uterus, thickness of endometrium and height of the endometrial epithelium.
All the animals in the treated groups showed open vagina, while all the control rats had closed vagina. Examination of the vaginal smears of the treated rats showed predominantly cornified and nucleated epithelial cells.

5.1.4 Discussion:

In the present study, the three extracts of the root of the *Momordica dioica* Roxb. were tested at the dose of 200 mg/kg p.o. All these extracts did not exhibit any anti-implantation activity. However, abortifacient activities were exhibited by these extracts. The aqueous extract showed 83%, whereas, the ethanolic extracts (soluble and insoluble parts) showed 100% abortifacient activity.

It is a well known fact that for implantation and sustainance of pregnancy, exact equilibrium of secretion of estrogen and progesterone is necessary. Any imbalance in the level of these hormones can cause anti-implantation or can induce abortion. Compounds disturbing hormonal functions may evoke infertility. In this study, the histological changes observed in the uterus of animals treated with various extracts supports an unfavourable uterine milieu. Therefore, the abortifacient activity may be due to mild estrogenic activity of the extracts causing expulsion or resorption of the implants by the uterus.
5.2 Androgenic/antiandrogenic effect of the ethanolic extract (soluble part) of the roots of *Momordica dioica* Roxb.

5.2.1 Introduction:

Many plants are known to contain chemical component/s which can affect fertility in males. Gossypol is a very potent male antifertility agent, isolated from the cotton plant. After discovery of this, many researchers have taken up screening of various plants and their part/s for evaluation of antifertility effect in males.

Literature survey has revealed that the aqueous extract of the root of *Momordica dioica* Roxb. has been known to possess spermicidal activity. So far this plant has not been studied for its male antifertility activity. Therefore, the present investigation has been undertaken to examine the possible androgenic/antiandrogenic effect of the ethanolic extract (soluble part) of the root of *Momordica dioica* Roxb.

5.2.2 Materials and Method:

Colony bred immature male albino rats, (Wistar strain) 22 days old and weighing between 28-32 g, were used for evaluation of androgenic or antiandrogenic activity. They were divided into four groups, each group comprising of six animals. The first group served as control and received the vehicle (Tween-80, 1%). The second group received testosterone suspension in 1% Tween-80 solution at the dose of 1 mg/kg subcutaneously. The third group received a suspension of the ethanolic extract soluble part in 1% Tween-80 at 200 mg/kg body weight. The fourth group received, in addition to testosterone, a test dose of the ethanolic extract (soluble part) at 200 mg/kg body weight p.o. All the treatments were given for seven days. On eighth day of the experiment, all the animals were sacrificed by decapitation. The testes, epididymes, vas deferens, seminal vesicle and ventral prostrate were dissected out, freed from surrounding
tissues and weighed quickly on a sensitive balance. Gravimetric changes were recorded and shown in Table-5.4.

Table-5.4: Effect of the ethanolic extract (soluble part) of the root of *Momordica dioica* Roxb. on the weights in mg of male reproductive organs when fed orally to immature male rats for seven days.

<table>
<thead>
<tr>
<th>Treatment (dose mg/kg)</th>
<th>Testis</th>
<th>Seminal vesicle</th>
<th>Ventral prostrate</th>
<th>Vas deferens</th>
<th>Epididymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>675.43±23.33</td>
<td>35.40±2.70</td>
<td>58.83±6.32</td>
<td>27.13±2.89</td>
<td>74.93±10.39</td>
</tr>
<tr>
<td>Testosterone (1 mg/kg)</td>
<td>417.33±20.23</td>
<td>78.83±1.83</td>
<td>96.14±7.21</td>
<td>43.28±4.02</td>
<td>90.43±7.03</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>599.47±18.93*</td>
<td>28.12±0.84*</td>
<td>31.23±3.89**</td>
<td>25.81±2.37*</td>
<td>69.41±3.01*</td>
</tr>
<tr>
<td>(soluble part) 200 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+testosterone 1 mg/kg</td>
<td>393.48±16.09**</td>
<td>60.84±1.63***</td>
<td>59.34±2.08*</td>
<td>37.73±2.63**</td>
<td>84.89±4.08*</td>
</tr>
</tbody>
</table>

* p < 0.1 when compared with control
** p < 0.01 when compared with control
*** p < 0.001 when compared with control
+ p < 0.5 when compared with control
++ p < 0.05 when compared with control

5.2.3 Results:

The administration of ethanolic extract (soluble part) of the root of *Momordica dioica* Roxb. to immature male rats for seven days has significantly reduced the weights of testes, epididymes, Seminal vesicle and ventral prostrate when compared with control. Simultaneous administration of the ethanolic extract (soluble part) and testosterone induced a reduction in the weights of all the organs when compared to testosterone administered animals. Considerable reduction in weights of Seminal vesicle and ventral prostrate were observed, thus indicating moderate antiandrogenic activity of the extract.
5.3 Anthelmintic Activity

5.3.1 Introduction:

Helminthiasis is a disease caused by infestation of parasitic worms living in the alimentary canal or in the other tissues of the host. This disease is one of the major prevalent diseases in the world, particularly in the tropical countries. The main reasons responsible for the widespread nature of this disease in the developing countries are the lack of adequate sanitary facilities and supply of water, coupled with poverty and illiteracy.

The helminth infection can be acquired by,

- Contact with infected animal
- The ingestion of infected meat
- The animal or human excreta via ground water
- Means of certain mosquitoes

The worms enter the body in the form of either larvae or egg. In some cases they produce diseases immediately, whereas in other cases they take weeks or months to produce diseases. Thus to take preventive measures against these worms, one should have exact understanding of the life cycle of helminthes. The main danger of helminthiases is that, the worms may cause injuries to the vessels and organs. They may cause loss of blood, nutritional deficiency, urticaria and other allergic conditions.

Anthelmintics are the drugs, which are used to kill or to remove the parasitic worms. Those anthelmintics, which kill the worm, are called vermicidal while those that help in expelling them by making the environment uncomfortable for living are called vermifuges.
An anthelmintic drug may act by causing narcosis or paralysis of worm either by
penetrating the cuticle or by the worm ingesting. It may damage the cuticle of the worm
or partially paralyse it. The specificity of anthelmintic reveals that they interfere with
metabolism of the worm, each worm is having its own metabolic requirement.

The human and animal parasitic worms zoologically belong to the class *Cestodes*
(Tapeworm), *Schistosomes* (Trematodes, flukes), *Filaria* (Threadworm), *Nematodes*
(Roundworms) and *Pheritina posthuma* (Earthworms, order-Annélida, class-
Oligochaeta).

Literature survey revealed that, researchers all over the world are engaged in
research work to synthesize compounds, which are capable of paralyzing these worms or
causing death. In animals and human beings, roundworms and tapeworms cause
sufficient trouble in the form of abdominal pain or crippling of movement. Therefore
scientific community has been making continuous effort to control menace of these
worms.

Fayard\textsuperscript{5} was the first scientist to discover piperazine, a heterocyclic compound, as
the drug having anthelmintic activity. Since then many derivatives of piperazine have
been synthesized in order to increase the efficacy of the drug and also to minimize side
effects. In addition, many other compounds have been synthesized, but only diethyl
carbazine could find place in human therapeutics.
The root of *Momordica dioica* Roxb. is reported to exhibit anthelmintic activity and henceforth it was contemplated to investigate the various extracts of the root for possible anthelmintic activity against earthworm.

As it was difficult to procure either roundworm or tapeworm, the experiment was performed on earthworms, which have similar life cycle.

Materials used, method adopted, observations made and results of screening are described in the following pages.

### 5.3.2 Materials and Method:

1. Earthworms (*Pheritima posthuma*)
2. Petridishes
3. Beakers and measuring jars
4. Standard drug (Piperazine citrate)
5. Glass rods
6. Stop watch

The technique adopted was that described by Gaind et al with slight modification.

Anthelmintic activity was evaluated by using earthworm *Pheritima Posthuma* (class-Annelida and order-Oligiocheata).

The worms were procured from local supplier at Shimoga at the time of carrying out the experiment. The worms were washed with water to remove adhering materials and were sorted out for uniform size and length. The worms were kept in 6% dextrose
solution for acclimatization. The worms with normal motility were selected for the experiment. Petridishes of equal size were selected and in each petridish, different concentrations i.e. 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml of the extracts in 0.1% Tween-80 suspension were placed and the volume was made upto 25 ml with an aqueous solution of dextrose (6%). In another petridish Tween-80 (25 ml, 0.1% solution) prepared in 6% dextrose solution was placed, which served as control, Piperazine citrate suspended in 0.1% Tween-80 in 6% dextrose solution of different concentrations, as mentioned above were prepared and placed in separate petridishes which served as standard. In each petridish 8 worms were placed. The time taken by each worm for paralysis was noted and the paralysis of worms was tested by placing the worms in water maintained at 50°C. Non-motility at 50°C was taken as the death of the worm and correspondingly the time taken for death was recorded [(Fig. 15, 16 and 16 (a)].

The results are tabulated in the Table-5.5.
Photograph showing the earthworms in dextrose solution (6%, control). Worms show normal motility.

Photograph showing the earthworms in the solution of ethanolic extract-soluble part of the root of *Momordica dioica* Roxb. in dextrose solution (6%) at 10 mg/ml concentration. Significant paralyzing effect of the extract is seen.
Anthelmintic Activity of Crude Extracts

Figure-16(a)  Extracts (10mg/ml)

- Piperazine Citrate
- Aqueous Extract
- Ethanolic Extract-Soluble part
- Ethanolic Extract-Insoluble part
Table 5.5: Effect of the extracts of *Momordica dioica* root on *Pheritima posthuma* worm

<table>
<thead>
<tr>
<th>Drug</th>
<th>Average % paralysis observed after 1 h±SEM</th>
<th>ED$_{50}$ mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mg/ml 4 mg/ml 6 mg/ml 8 mg/ml 10 mg/ml</td>
<td></td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>25.0 52.0±2.08 64.5±2.08 75.0 85.4±3.84 3.802</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>29.1±2.64* 54.1±2.64* 70.8±2.60* 81.3±2.79 91.6±2.63* 3.467</td>
<td></td>
</tr>
<tr>
<td>Ethanolic extract soluble part</td>
<td>20.8±2.63* 39.5±2.08 52.0±2.08 58.3±2.63 64.5±1.8 5.623</td>
<td></td>
</tr>
<tr>
<td>Ethanolic extract insoluble part</td>
<td>22.9±2.08* 43.75±2.79* 56.25±2.79* 64.5±1.8 70.8±2.63* 4.898</td>
<td></td>
</tr>
</tbody>
</table>

p<0.5 vs. piperazine citrate, student’s 't'-test, n=8

5.3.3 Results:

Various extracts of the root of *Momordica dioica* Roxb. exhibited significant anthelmintic activity against earthworms. Maximum paralytic activity was observed at 8 mg/ml and 10 mg/ml concentration and it was comparable with standard piperazine citrate. Aqueous extract showed equipotent anthelmintic activity at 8 mg/ml and 10 mg/ml concentration when compared with piperazine citrate at the same dose.

5.3.4 Discussion:

Earthworms have the ability to move by ciliary movement. Outer layer of the earthworm is a mucilaginous layer and composed of polysaccharides. This layer, being
slimy, enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layers, and this restricts its movement and can cause paralysis. This action may lead to the death of the worm.

The drugs possessing anthelmintic property will paralyse the worms by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis. Since, the worm is incapable of movement, it will be expelled out from the body. The anthelmintic activity of the various extracts may be attributed to the similar type of action.
5.4 Anti-inflammatory Activity

5.4.1 Introduction:

Inflammation is a normal, essential protective response to any noxious stimulus that may threaten the host and may vary from a localized reaction to a complete response involving the whole organism. Rheumatic diseases are inflammatory conditions that probably cause more disability than any other group of diseases and are very common among persons with age more than forty years.

In ordinary conditions the inflammatory and reparative processes, progress smoothly from injury to healing and here the whole process is beneficial under special circumstances. Under special circumstances, eosinophils may concentrate in large number in tissue leading to restoration, as in the case of acute inflammation. In contrast many chronic inflammatory conditions of unknown etiology, which affect organ system in the body and produce tissue destruction rather than restoration. Chronic inflammatory reactions can be divided into two groups, those with and without the formation of granuloma depending on the agent responsible for inflammation. When the irritant cannot be easily disposed by the mononuclear phagocytes, granuloma formation unusually occurs. Such inflammation may become much more complex and inflammatory response try to isolate them from the rest of the organisms by forming granuloma or pus, this is the case which is observed in pulmonary tuberculosis and in syphilis. Apart from these, there are various chronic inflammatory conditions of unknown etiology like rheumatoid arthritis, spondylitis, gout etc., Anti-inflammatory drugs are the drugs used to diminish or to reduce inflammation and pain arising from it. They modify the inflammatory response to disease. So an ideal anti-inflammatory drug should only affect
the aberrant, uncontrolled inflammation and should not interfere with the normal inflammatory system. Anti-inflammatory drugs have been evaluated by studying inflammatory responses produced in animals by injecting foreign or noxious agents. These responses mostly comprise of the development of edema.

It is reported in the literature that the fruit of the plant *Momordica dioica* Roxb. has been used to treat the inflammation caused by excretion of lizards. Hence, it is contemplated to take up the extracts of the root of this plant for a detailed study for anti-inflammatory properties.

Among several models available for evaluating anti-inflammatory activity, the rat paw edema model is the simplest and widely used method for this purpose.

The inflammatory reaction is readily produced in rats in the form of paw edema with the help of irritants or inflamagens. Substances such as carrageenan, formalin, bradykinin, histamine, 5-hydroxy tryptamine etc., produce acute paw edema within a few minutes of the injection. Carrageenan induced paw edema is the most commonly used method in experimental pharmacology.

5.4.2 Materials and method:

1. Albino rats weighing 150-200 g of Wistar strain
2. Plethysmometer
3. Beakers and measuring jar
4. Standard drug (Ibuprofen)
5. Potassium permanganate solution (0.1%)
6. Tween-80 solution (0.1%)
7. Carrageenan (0.1%)
Rat paw edema method

This method is based on plethysmographic measurement of carrageenan-induced acute rat paw edema°-14 produced by subplantar injection of carrageenan in hind paw of the rat. The method described by Wilhlmi and Demenjoz15, later modified by Sirodia and Rao16 was used for measuring the paw volume17-21. The photographs of the measurement of rat paw volume are given in Fig. 17-20 and 20(a) shows bar diagram.

This procedure of measuring the paw volume required two operators, one for dipping the paw of the rat in potassium permanganate solution and another to measure and record the paw volume simultaneously.

Before measuring the paw volume of the rat, the volume of solution in graduated tube ‘F’ was set to zero by operating the side tube ‘G’, after filling the apparatus with potassium permanganate solution without any entrapment of air bubble. The paw was dipped in limb ‘A’ up to the line marked on the foot of the rat by keeping the stopcock ‘C’ open and ‘E’ closed. The first operator held the foot of the rat firmly in potassium permanganate solution till the stopcock ‘C’ was kept closed by the second operator. The solution in the limb ‘B’ rises and now the stopcock ‘C’ was kept closed. The stop cock ‘E’ was slowly opened to bring back the raised solution in limb ‘B’ so as to bring the level of solution equal in limbs ‘A’ and ‘B’.

Immediately the stopcock ‘E’ was closed. The solution rises in the graduated part ‘E’ of the apparatus and the increase in volume of the solution was noted. Now the side limb ‘G’ was opened to remove the solution in the graduated tube to bring it to zero and immediately the stopcock ‘G’ was closed. The stopcock ‘D’, meant for removing the air
Photograph showing the extract being injected in the right hind paw for inducing edema.

Photograph showing the inflamed right hind paw.
Figure-19  Photograph showing the plethysmometer used for measuring paw edema.

Figure-20  Photograph showing the inflamed right hind paw of the rat being dipped in the plethysmometer for measuring the paw volume.
bubble in limbs ‘A’ and ‘B’, was kept closed throughout the experiment. This procedure was repeated every time for measuring the paw volume.

For this study, albino rats (Wistar strain) of either sex weighing between 100-200 g were used and divided into 5 groups of 6 animals each. The group I served as control and received 1 ml of 0.1% Tween-80 solution orally. The group II received ibuprofen in Tween–80 (0.1%) at a dose of 200 mg/kg body weight and served as standard. The groups III, IV and V received orally the various extracts (aqueous, ethanolic-soluble and insoluble part) at the dose of 200 mg/kg body weight in Tween-80 (0.1%)solution. These drugs were administered one hour before the injection of an irritant, carrageenan. After 1 hour all the animals were injected subcutaneously 0.1% of carrageenan (0.05 ml) solution to the right hind paw in the subplantar region and immediately the paw volume was measured. After 3 hours the paw volume was measured in control, in standard and in test groups. The results are given in Table-5.6.
Table-5.6: Anti-inflammatory activity of ethanolic extract-soluble and insoluble parts of the root of *Momordica dioica* Roxb.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Increase in paw volume after 3 h (ml) ±SEM</th>
<th>% decrease in paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.1% Tween 80, 1 ml)</td>
<td>1.26±0.213</td>
<td>--</td>
</tr>
<tr>
<td>Ibuprofen (200 mg/kg in 0.1% Tween 80)</td>
<td>0.44±0.064</td>
<td>65.0*</td>
</tr>
<tr>
<td>Aqueous extract (200 mg/kg in 0.1% Tween 80)</td>
<td>0.61±0.020</td>
<td>51.7*</td>
</tr>
<tr>
<td>Ethanolic extract-soluble part (200 mg/kg in 0.1% Tween 80)</td>
<td>0.61±0.023</td>
<td>51.6*</td>
</tr>
<tr>
<td>Ethanolic extract-insoluble part (200 mg/kg in 0.1% Tween 80)</td>
<td>0.59±0.028</td>
<td>53.0*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM., n=6 * p<0.001 compared with control

The percentage inhibition of paw volume was calculated by using the formula

\[
\left(1 - \frac{V_t}{V_c}\right) \times 100,
\]

Where

- \(V_t\) = Mean increase in the paw volume in test animals group
- \(V_c\) = Mean increase in the paw volume in control group.

Statistical analysis was carried to determine % protection.
Standard Deviation (S.D.) and Standard Error Mean (SEM.) were calculated by using the following equation.

\[
S.D. = \left( \frac{\Sigma d^2 - \frac{(\Sigma d)^2}{n}}{n - 1} \right)^{\frac{1}{2}}
\]

\[
SEM. = \frac{S.D.}{\sqrt{n}}
\]

Where 'n' = Number of animals in each group (6).

'd' = Increase in paw volume in Albino rats.

Calculation of SD, SEM and % protection for aqueous extract of the root of *Momordica dioica* Roxb. is shown in Table-5.7.

**Table-5.7:** Statistical data for aqueous extract of the root of *Momordica dioica* Roxb.

<table>
<thead>
<tr>
<th>Albino rat</th>
<th>Difference in paw volume after 3 h in control group</th>
<th>Square of the paw difference</th>
<th>Difference in paw volume after 3 h in test group</th>
<th>Square volume after 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.40</td>
<td>1.96</td>
<td>0.65</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
<td>1.40</td>
<td>1.96</td>
<td>0.60</td>
<td>0.36</td>
</tr>
<tr>
<td>3</td>
<td>0.85</td>
<td>0.72</td>
<td>0.65</td>
<td>0.42</td>
</tr>
<tr>
<td>4</td>
<td>1.10</td>
<td>1.21</td>
<td>0.65</td>
<td>0.42</td>
</tr>
<tr>
<td>5</td>
<td>1.40</td>
<td>1.96</td>
<td>0.60</td>
<td>0.36</td>
</tr>
<tr>
<td>6</td>
<td>1.40</td>
<td>1.96</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7.55</strong></td>
<td><strong>9.77</strong></td>
<td><strong>3.65</strong></td>
<td><strong>2.23</strong></td>
</tr>
</tbody>
</table>
Statistical calculation for control group,

\[
\Sigma d = 7.55
\]

\[
\Sigma d^2 = 9.77
\]

\[
S.D = \sqrt{\frac{9.77 - \frac{(7.55)^2}{6}}{6 - 1}}
\]

\[
S.D = \sqrt{9.77 - 9.50}
\]

\[
S.D = \sqrt{0.27}
\]

\[
S.D = 0.23
\]

\[
SEM = \frac{0.23}{\sqrt{6}} = 0.095
\]

Mean increase in rat paw volume in control group = \(\frac{7.55}{6} = 1.26\)

Mean increase in paw volume = 1.26±0.095
Statistical calculation for aqueous extract,

\[ \Sigma d = 3.65 \]

\[ \Sigma d^2 = 2.33 \]

\[
S.D = \left( \frac{2.23 - \frac{(3.65)^2}{6}}{6 - 1} \right)^{\frac{1}{2}}
\]

\[
S.D = \left( \frac{2.23 - 2.22}{5} \right)^{\frac{1}{2}}
\]

\[ S.D = \left( 0.002 \right)^{\frac{1}{2}} \]

\[ S.D = 0.45 \]

\[
SEM = \frac{0.45}{\left( \frac{6}{\sqrt{6}} \right)^{\frac{1}{2}}} = 0.02
\]

Mean increase in rat paw volume in aqueous extract \( \frac{3.65}{6} \) = 0.608

Mean increase in paw volume \( 0.608 \pm 0.02 \)

\[
\% \text{ Protection} = \left( 1 - \frac{0.608}{1.26} \right) \times 100
\]

\[ = 51.7\% \]
Percentage protection for other extracts has been calculated by similar method and recorded in Table-5.6.

<table>
<thead>
<tr>
<th>Model</th>
<th>Acute inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Carrageenan induced edema</td>
</tr>
<tr>
<td>Animals</td>
<td>Albino rats (Wistar strain)</td>
</tr>
<tr>
<td>Number of animals in each group</td>
<td>6</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Oral</td>
</tr>
<tr>
<td>Standard</td>
<td>Ibuprofen (200 mg/kg.)</td>
</tr>
<tr>
<td>Extracts</td>
<td>Aqueous (200 mg/kg.)</td>
</tr>
<tr>
<td></td>
<td>Ethanol-soluble part (200 mg/kg.)</td>
</tr>
<tr>
<td></td>
<td>Ethanol-insoluble part (200 mg/kg.)</td>
</tr>
<tr>
<td>Control</td>
<td>Tween-80 (0.1%)</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
</tbody>
</table>

5.4.3 Results:

Various extracts of the roots of *Momordica dioica* Roxb. were tested for anti-inflammatory activity against carrageenan – induced rat paw edema. The activity was compared with ibuprofen. Aqueous extract, ethanolic extract (soluble part and insoluble part) showed 51.7%, 51.6% and 53% inhibition respectively as against standard ibuprofen which showed 65% inhibition after 3 h.
5.4.4 Discussion:

The results clearly indicate that various extracts possess anti-inflammatory activity.

Carrageenan-induced rat paw edema model is commonly used for evaluating anti-inflammatory activity. The effect is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in first hour after administration of carrageenan and a more pronounced second phase, is attributed to the release of bradykinin, protease, prostaglandin and lysozyme. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents. The results suggest that mechanism of action of the tested extracts required more investigation.
5.5 Analgesic Activity

5.5.1 Introduction

In early days, the mankind depended on divine healing through laying on of hands or prayer for relief of pain. However, after sometime man did not rely exclusive on spiritual methods for pain reduction. Portions concocted from herbal extracts were used by earlier physicians for this purpose. Alcohol and ethanolic extract of opium were used by Hippocrates for the production of the insensibility to pain. It was only when the first alkaloid morphine, isolated in 1803 by Sertumer the scientific, era of analgesia was realized. Analgesia can be defined as a reduction of pain perception without the loss of consciousness. The diminution of pain may be a consequence of febrifuge effect of the drug. After the discovery of morphine as an analgesic, lot of research work has been carried out to increase the efficacy of morphine and reduce its undesired side effects. The research work mainly was on the following lines.

- Structural modification of morphine
- Synthesis of morphine like compounds
- Synthesis of other heterocyclic compounds
- Random screening of synthesized compounds
- Subjecting different plant extracts for analgesic response on mice or rats
- Isolation of active components from plant extracts

It is a well known fact that plant possessing anti-inflammatory property can act as analgesic also. Literature survey has revealed that the juice of the fruit of this plant possesses anti-inflammatory activity in the treatment of inflammation caused by excretion of lizards. As various extracts of the root of the *Momordica dioica* Roxb. has
been shown to possess significant anti-inflammatory activity, it is envisaged now to subject various extracts for analgesic activity in albino mice.

In the present investigation, analgesic activity of various extracts was evaluated by using the first two methods.

Method adopted, materials used and results are discussed in the following paragraphs.

There are mainly three methods available to evaluate analgesic activity namely,

a. Acetic acid induced abdominal constriction method in mice
b. Tail flick method in rats
c. Eddy’s hot plate analgesiometer method in mice

5.5.2 Acetic acid induced abdominal constriction method:

Materials:

1. Albino mice weighing 22-35 g of Swiss strain
2. Acetic acid (0.6%)
3. Marker pens
4. Standard drug (Acetyl salicylic acid)
5. Stop clock
6. Tween–80 solution (0.1%)

Colony bred albino mice (Swiss strain) of either sex weighing 22-35 g were used to evaluate analgesic activity. Analgesic activity was determined as described by the method based on acetic acid induced writhing in mice. The photographs showing analgesic activity are appended in Fig. 21, 22 and 22(a).
Figure-21  Photograph showing the rat being injected with acetic acid for the induction of writhing.

Figure-22  Photograph showing the rat exhibiting writhing.
For this experiment, 30 mice were used and they were divided into 5 groups containing 6 animals in each group. All the animals received 0.6% acetic acid solution (10 ml/kg body weight) intraperitoneally and numbers of writhing were recorded after 5 min. for the period of 20 min. These groups served as control group. The same groups of animals were used next day for evaluating analgesic activity of various extracts of the plant.

First group received Tween-80 (0.5 ml, 0.1%) and served as control, second group received 150 mg/kg body weight of acetyl salicylic acid (aspirin) orally and served as standard. The animals in third, fourth and fifth groups received various extracts of the root of *Momordica dioica* Roxb. at a dose of 200 mg/kg p.o. suspended in Tween-80 (0.1%). After 1 h, all the animals received 10 ml/kg body weight of acetic acid (0.6%) intraperitoneally, and number of writhing were recorded after 5 min for 20 min. The results are recorded in the Table-5.8.
Table-5.8: Effect of *Momordica dioica* extracts on acetic acid induced writhing response in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose mg/kg</th>
<th>Mean no. of writhings occurred between 5 and 15 min. ±SEM</th>
<th>%MPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control 5 ml Water</td>
<td>45.83±1.28</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>II</td>
<td>ASA 150</td>
<td>11.83±1.3*</td>
<td>74.19</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Aqueous extract 200</td>
<td>22.16±1.9*</td>
<td>51.65</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Ethanolic extract (soluble part) 200</td>
<td>18.17±2.2*</td>
<td>60.35</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Ethanolic extract (Insoluble part) 200</td>
<td>08.50±1.1*</td>
<td>81.45</td>
<td></td>
</tr>
</tbody>
</table>

* p<0.001 vs control, student’s 't'-test, n=6.

The percentage of protection was calculated by using the formula

\[(1-V_t/V_c) \times 100,\]

Where \(V_t = \text{Mean number of writhing in test animals}\)

\(V_c = \text{Mean number of writhing in control.}\)

Statistical analysis was carried to determine % protection.

Standard deviation (S.D) and Standard Error Mean (SEM) were calculated by using the following equation,

\[S.D = \left( \frac{\Sigma d^2 - (\Sigma d)^2}{n} \right)^{\frac{1}{2}} \]

\[\frac{n-1}{\text{S.D}}\]
SEM = \frac{\text{S.D}}{\left( \frac{n}{n}\right)^{\frac{1}{2}}}

When ‘n’ = Number of animals in each group (6).

‘d’ = Number of writhing in mice.

The number of writhing before administration of drug and after administration of drug, for aqueous extract is as shown in the following Table-5.9. Calculation of S.D, SEM and % protection is shown in detail in the following paragraphs.

Table-5.9: Statistical data for aqueous extract of the root of Momordica dioica Roxb.

<table>
<thead>
<tr>
<th>Mice</th>
<th>No. of writhing before administration of drug</th>
<th>Square of the No. of writhing</th>
<th>No. of writhing after administration of drug</th>
<th>Square of the No. of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>1936</td>
<td>25</td>
<td>625</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>1681</td>
<td>19</td>
<td>361</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>2116</td>
<td>18</td>
<td>324</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>2304</td>
<td>21</td>
<td>441</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>2116</td>
<td>20</td>
<td>400</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>2500</td>
<td>30</td>
<td>900</td>
</tr>
<tr>
<td>Total</td>
<td>275</td>
<td>12,653</td>
<td>133</td>
<td>3051</td>
</tr>
</tbody>
</table>
Statistical calculation for, before administration of drug, in control group,

\[ \Sigma d = 275 \]
\[ \Sigma d^2 = 12,653 \]

\[
S.D = \left( \frac{12653 - \left(\frac{(275)^2}{6}\right)}{6-1} \right)^{\frac{1}{2}}
\]

\[
S.D = \left( \frac{12653 - 12604}{5} \right)^{\frac{1}{2}}
\]

\[ S.D = 3.13 \]

\[
SEM = \frac{3.13}{\sqrt{6}} = 1.28
\]

Mean number of writhing = \[ \frac{275}{6} \] = \[ 45.83 \]

Mean writhing = \[ 45.83 \pm 1.28 \]

Statistical calculation for, after administration of drug in test group

\[ \Sigma d = 133 \]
\[ \Sigma d^2 = 3051 \]
S.D = \left( \frac{3051 - \frac{(133)^2}{6}}{6 - 1} \right)^{\frac{1}{2}}

\[
S.D = \left( \frac{3051 - 2948}{5} \right)^{\frac{1}{2}}
\]

S.D = \left( 20.6 \right)^{\frac{1}{2}}

S.D = 4.54

SEM = \frac{4.54}{\left( \frac{6}{5} \right)^{\frac{1}{2}}} = 1.85

Mean number of writhing = \frac{133}{6} = 22.16

\[
\text{Mean writhing} = 22.16 \pm 1.85
\]

% Protection = \left( 1 - \frac{22.16}{45.83} \right) \times 100

% Protection = 51.65%

By similar method, S.D, SEM and % protection for all other extracts were calculated.
5.5.3 Tail Flick Method:

Materials:

1. Albino rats weighing between 150 to 200 g (Wistar strain)
2. Marker pens
3. Standard drug (Acetyl salicylic acid)
4. Stop clock
5. Tween-80 solution (0.1%)

Method described by Ghosh\textsuperscript{33} and Kulkarni\textsuperscript{34} was adopted. Albino rats (Wistar strain) of either sex, weighing between 150-200 g were divided into 5 groups, each group comprising of 6 animals. All the animals were fed with standard diet and water \textit{ad libitum} and fasted for 24 h prior to the experiment. First group served as control and received 1% Tween-80 (1ml p.o.). Second group served as standard and received acetyl salicylic acid suspended in 1% Tween-80 at a dose of 200 mg/kg p.o., whereas, animals in third, fourth and fifth groups received crude extracts at the same dose suspended in Tween-80 (1%). Tail of each animal was placed in water at 50\degree C\pm 1\degree C and the time of withdrawal of tail was recorded before the commencement of the test and it was noted as an Average Basal Reaction Time (ABRT). The time of tail withdrawal (in seconds) were recorded at 30, 60, 90, 120, 180 and 240 min. in all the animals in each group and the results are tabulated in Table-5.10.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pretreatment (0 min)</th>
<th>30 min.</th>
<th>60 min</th>
<th>90 min.</th>
<th>120 min.</th>
<th>180 min.</th>
<th>240 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1% Tween-80</td>
<td>2.2</td>
<td>2.30</td>
<td>2.30</td>
<td>2.20</td>
<td>2.30</td>
<td>2.30</td>
<td>2.00</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>2.6</td>
<td>3.33 ± 0.21</td>
<td>3.50 ± 0.22</td>
<td>4.00 ± 0.26</td>
<td>5.33 ± 0.21</td>
<td>7.83 ± 0.17</td>
<td>2.67 ± 0.21</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>2.1</td>
<td>2.58 ± 0.22</td>
<td>2.75 ± 0.31</td>
<td>2.92 ± 0.38</td>
<td>3.70 ± 0.21*</td>
<td>6.33 ± 0.33*</td>
<td>3.17 ± 0.31</td>
</tr>
<tr>
<td>Ethanol extract soluble part</td>
<td>2.1</td>
<td>2.25 ± 0.17</td>
<td>2.50 ± 0.22</td>
<td>3.17 ± 0.17</td>
<td>3.30 ± 0.21*</td>
<td>5.33 ± 0.33*</td>
<td>2.50 ± 0.22</td>
</tr>
<tr>
<td>Ethanol extract insoluble part</td>
<td>2.0</td>
<td>2.58 ± 0.20</td>
<td>3.25 ± 0.49</td>
<td>4.17 ± 0.17*</td>
<td></td>
<td></td>
<td>3.70 ± 0.51</td>
</tr>
</tbody>
</table>

*P < 0.001 vs Control, student's t-test, n=6
5.5.4 Results:

The observation made in both the experiments revealed that ethanolic extract-insoluble part was more active than the remaining two extracts. Aqueous extract and ethanolic extract-soluble part showed moderate analgesic response when compared with standard drug. In Tail Flick method, it was observed that analgesic activity of the extracts and standard drug diminished after 180 min.

5.5.5 Discussion:

Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nerves system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptor site of pain, while centrally acting analgesics not only rise the threshold of pain, but also alter the physiological response to pain and suppress the patient's anxiety and apprehension. Various extracts of the root of Momordica dioica Roxb. tested for analgesic activity by writhing method and tile flick method have shown significant analgesic activity when compared with acetyl salicylic acid. The activity is attributed to the presence of triterpenoid saponin glycoside. It was well supported by the fact that the extracts possess significant anti-inflammatory activity as inflammation and pain co-exists together.
5.6 Anticatatonic Activity

5.6.1 Introduction:

It is well known fact that many drugs produce extra pyramidal side effects i.e., catatonia in animals. These effects, such as, akinesia, rigidity and tremors, are reflected in the form of major clinical symptoms like difficulty to move, change in posture etc. Hence, it is felt necessary and interesting to study catatonic or anticatatonic effect of crude extracts of the root of Momordica dioica Roxb.

5.6.2 Materials and Method:

1. Albino rats (Wistar strain) 150–200 g
2. Scopolamine (Standard drug)
3. Tween-80 solution.
4. Wooden blocks of 3 cm and 9 cm height.
5. Chlorpromazine

Anticatatonic activity of the various extracts was carried out by the method described by Kulkarni\textsuperscript{35} and Dandia\textsuperscript{36}.

Rats of either sex weighing between 150-200 g were fasted for 24 h with water ad libitum. They were divided into 5 groups consisting of 6 animals in each group. The first group served as control and received the vehicle Tween-80 (1%, 1 ml). The second group received scopolamine at the dose of 2 mg/kg suspended in 1% Tween-80 solution intraperitoneally, which served as standard. Third, fourth and fifth groups received aqueous extract, ethanolic extract-soluble part and ethanolic extract-insoluble part at the dose of 200 mg/kg p.o. suspended
in Tween-80 (1%) solution. To induce catatonia in animals, chlorpromazine at the dose of 5 mg/kg i.p. was injected into each animal 1 h after the drug treatment.

The four stages of catatonia and corresponding scores are shown in Table-5.11. The severity of catatonia was observed in all the animals at 5, 15, 30, 45, 60, 90, 120 and 180 minutes after chlorpromazine injection. The results are tabulated in Table-5.12.

Statistical analysis was carried out by student’s ‘t’ test and the results are expressed as ±SEM.

Table-5.11: Four stages of catatonia and score

<table>
<thead>
<tr>
<th>Stage</th>
<th>Condition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Rat moved normally when placed on the table.</td>
<td>0.0</td>
</tr>
<tr>
<td>II</td>
<td>Rat moved normally when touched or pushed</td>
<td>0.5</td>
</tr>
<tr>
<td>III</td>
<td>Rat placed on the table with the front paws set alternatively on a 3 cm height block failed to correct the posture in 10 seconds, 0.5 for each paw</td>
<td>1.0</td>
</tr>
<tr>
<td>IV</td>
<td>Rat failed to remove the front paws when placed on a 9 cm height block. 1 for each paw.</td>
<td>2.0</td>
</tr>
<tr>
<td>Treatment and dose</td>
<td>Severity of catatonia after (mean ±SEM)</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Scopolamine (2 mg/kg)</td>
<td>0</td>
<td>0.16±0.10*</td>
</tr>
<tr>
<td>Aqueous extract (200 mg/kg.)</td>
<td>0</td>
<td>0.67±0.11</td>
</tr>
<tr>
<td>Ethanol extract soluble part (200 mg/kg)</td>
<td>0</td>
<td>0.75±0.11</td>
</tr>
<tr>
<td>Ethanol extract insoluble part (200 mg/kg)</td>
<td>0</td>
<td>0.83±0.11</td>
</tr>
</tbody>
</table>

*p<0.001 vs control, student's 't' test, n = 6.
5.6.3 Results:

All the animals, which received the extracts, did not score significantly when compared with scopolamine. This indicated that none of the extracts has anticatatonic property.
5.7 Diuretic Activity

5.7.1 Introduction:

Diuretics are class of drugs that increase the rate of urine flow, however clinically useful diuretics also increase the rate of excretion of Na⁺ and an accompanying anion, usually, Cl⁻. Sodium chloride in the body is the major determinant of extracellular fluid volume and most clinical applications of diuretics are directed towards reducing extracellular fluid volume by decreasing total body sodium chloride content. A sustained imbalance between dietary sodium loss was incompatible with life. A sustained positive sodium balance would result in volume overload with pulmonary edema and a sustained negative sodium balance would result in volume depletion and cardiovascular collapse. Although, continued administration of a diuretic causes sustained net deficit in total body sodium, the time course of natriuresis was finite as renal compensatory mechanisms bring sodium excretion in line with sodium intake, a phenomenon known as diuretic braking. Frusemide is a standard drug, used to evaluate diuretic activity.

5.7.2 Materials and Method:
1. Albino rats weighing 150-200 g of (Wistar strain)
2. Tween-80 solution (0.1%)
3. Stop clock
4. Standard drug (Frusemide)
5. Metabolic cage
6. Beaker
7. Measuring jar
For evaluation of diuretic activity in albino rats (Wistar strain), the method adopted by Lipschitz was employed. Rats of either sex, weighing between 100-200 g were divided into 5 groups each containing six animals. All the animals were fasted for 24 h prior to the test and received a primary dose of saline 25 ml/kg body weight orally. The first group served as control and received Tween–80 (0.1%, 5 ml) in distilled water orally. The second group received frusemide suspension in Tween–80 (0.1%) at the dose of 40 mg/kg body weight orally and this group served as standard. The third, fourth and fifth groups received extracts (aqueous, ethanolic–soluble part and ethanolic–insoluble part) at the dose of 200 mg/kg body weight orally suspended in Tween–80 (0.1%) (Fig. 23). Saline (aqueous solution 0.9%), at the dose of 25 ml/kg body weight was administered orally to all the animals and were placed in metabolic cages (Fig. 24) (4 rats per cage) immediately after the administration of the extracts. Urine samples, collected in 5 h time from each group, were pooled and volume was measured. pH of urine samples were measured using a single electrode pH meter. The results are tabulated in Table-5.13.
Figure-23 Photograph showing the rat receiving the injection of the aqueous extract of the root of *Momordica dioica* Roxb. for envisaging diuretic activity.
Figure-24  Photograph showing the rats in metabolic cage.
Anti-inflammatory Activity

![Graph of Anti-inflammatory Activity](image)

Figures 20(a) and 21(a) show the percentage decrease in paw volume of extracts (200 mg/kg) compared to Ibuprofen, Aqueous Extract, Ethanol Extract-Soluble part, and Ethanol Extract-Insoluble part.

Analgesic Activity

![Graph of Analgesic Activity](image)

Figures 22(a) and 23(a) illustrate the percentage MPE of extracts (200 mg/kg) compared to Acetyl salicylic acid, Aqueous Extract, Ethanol Extract-Soluble part, and Ethanol Extract-Insoluble part.
Table-5.13: Diuretic activity of various extracts of the root of *Momordica dioica* Roxb.

<table>
<thead>
<tr>
<th>Treatment (dose mg/kg)</th>
<th>No. of animals</th>
<th>Volume of urine (collected after 5 h in ml)</th>
<th>T/S (Lipschitz values)</th>
<th>Mean urine pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>33</td>
<td>--</td>
<td>8.21</td>
</tr>
<tr>
<td>Frusemide (40 mg/kg)</td>
<td>6</td>
<td>17</td>
<td>0.52</td>
<td>7.07</td>
</tr>
<tr>
<td>Aqueous extract (200 mg/kg)</td>
<td>6</td>
<td>18</td>
<td>0.55</td>
<td>7.1</td>
</tr>
<tr>
<td>Ethanolic extract (Soluble part) 200 mg/kg</td>
<td>6</td>
<td>20</td>
<td>0.61</td>
<td>7.09</td>
</tr>
<tr>
<td>Ethanolic extract (Insoluble part) 200 mg/kg</td>
<td>6</td>
<td>18</td>
<td>0.55</td>
<td>7.12</td>
</tr>
</tbody>
</table>

Index for diuretic activity

- Animals: Albino rats
- No. of animals per group: 6
- Standard: Frusemide
- T: Urine collected for test compound in ml
- S: Urine collected for standard drug in ml
- Control: Tween-80

5.7.3 Results:

The mean urine volume and pH of various extracts of the root of *Momordica dioica* Roxb. was almost same when compared with control group. The Lipschitz value for the standard drug Frusemide is taken as 1. The tested extracts showed less T/S value. Hence, the extracts are devoid of diuretic activity.
5.8 Hepatoprotective Activity

5.8.1 Introduction:

Liver disorders may be classified as hepatitis (inflammation of the liver), hepatosis (non-inflammatory disorders or degeneration of the liver parenchyma), chronic hepatitis and liver cirrhosis. It is impossible to classify hepatoprotective agents as the preparations used against liver disorders does not possess specific activity. Hence, we find about 600 commercial preparations against hepatitis alone. It must, therefore, be concluded that an actual curative and therapeutic agent has not yet been found. Most preparations rather support/promote the process of healing or regeneration of the liver. In this context, antihepatotoxic activity is especially important.

Many indigenous plants and formulations have been claimed to possess hepatoprotective activity.

The plants having significant hepatoprotective action against Carbon tetrachloride, Paracetamol and Alcohol induced liver damage are listed in Table-5.14.

Table-5.14: Plants having hepatoprotective activity

<table>
<thead>
<tr>
<th>Name</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picrorrhiza kurroa</td>
<td>40,41,42</td>
</tr>
<tr>
<td>Andrographis paniculatus</td>
<td>43,44</td>
</tr>
<tr>
<td>Phyllanthus niruri</td>
<td>45</td>
</tr>
<tr>
<td>Eclipta alba</td>
<td>41,46,47</td>
</tr>
<tr>
<td>Piper longum</td>
<td>48</td>
</tr>
</tbody>
</table>
As part of the present investigation on the root of *Momordica dioica* Roxb., ethanolic extract-soluble part of the root, was screened for possible hepatoprotective activity.

5.8.2 Materials and Method:

The method reported by Chandra et al., is adopted for the evaluation of this activity. Adult male albino rats weighing between 200-250 g were selected. They were housed in polypropylene cages in well-ventilated rooms with exhaust fans. All the animals were fed with standard rat feed.

Group I (Control)

Animals were treated with aqueous solution of Tween-80 (0.1%, 0.2 ml) for 7 days. Blood samples were collected by direct heart puncture on 8th day to estimate serum enzyme levels of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and alkaline phosphatase. The animals were sacrificed, liver excised off and kept in formalin (10%) for histopathological studies.

Group II (Standard)

All the animals of this group were treated with Carbon tetrachloride (0.4 ml/kg body weight) for seven days. Then the animals were administered orally with the suspension of Sylimarin in aqueous solution of Tween-80 (0.1%) at the dose of 1.25 mg/kg body weight from 8th day to 14th day. Blood samples were withdrawn on 15th day to estimate the level SGOT, SGPT and alkaline
phosphatase in the serum. The animals were sacrificed for histopathological studies of the liver.

Group III (Extract)

All the animals of this group were treated with carbon tetrachloride (0.4 ml/kg body weight) for seven days. Then the animals were administered orally with the suspension of ethanolic extract-soluble part in aqueous solution of Tween-80 (0.1%) at the dose of 200 mg/kg body weight from 8th day to 14th day. Blood samples were withdrawn on 15th day to estimate the level SGOT, SGPT and alkaline phosphatase in the serum. The animals were sacrificed for histopathological studies of the liver.

Group IV (Carbon tetrachloride)

Animals in this group were given carbon tetrachloride daily at the dose of 0.4 ml/kg body weight i.p., for seven days. Blood samples were withdrawn on 8th day to estimate serum enzyme levels. Animals were sacrificed for histopathological studies of the liver.

The results are shown in Tables-5.15 and 5.16.
### Table-5.15: Histopathalogy of the liver

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatment</th>
<th>Dose</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Control Tween-80 (0.1%)</td>
<td>0.2 ml p.o.</td>
<td>Normal hepatocyte, sinusoid and portal tract (Fig. 25)</td>
</tr>
<tr>
<td>02</td>
<td>Sylimarin</td>
<td>1.25 mg/kg p.o.</td>
<td>Normal hepatocyte and mild inflammation (Fig. 26)</td>
</tr>
<tr>
<td>03</td>
<td>Ethanolic extract-soluble part</td>
<td>200 mg/kg</td>
<td>Mild congestion and inflammation (Fig. 27)</td>
</tr>
<tr>
<td>04</td>
<td>CCl₄</td>
<td>0.4 ml/kg i.p.</td>
<td>Fatty change around portal tract, hepatocyte with fatty changes and shows peripherally pushed nuclei. (Fig. 28)</td>
</tr>
</tbody>
</table>

### Table-5.16: Effect of hepatogard on serum enzyme level of Carbon tetrachloride treated albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Compounds and dose</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>Alkaline Phosphatase (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ±S.E</td>
<td>Mean ±S.E</td>
<td>Mean ±S.E</td>
</tr>
<tr>
<td>01</td>
<td>Control</td>
<td>50.17±2.57</td>
<td>45.83±2.65</td>
<td>429.83±3.81</td>
</tr>
<tr>
<td>02</td>
<td>Sylimarin 1.25 mg/kg</td>
<td>58.83±1.70</td>
<td>63.0±1.29</td>
<td>423.67±3.18</td>
</tr>
<tr>
<td>03</td>
<td>Ethanolic extract-soluble part 200 mg/kg</td>
<td>85.33±2.76</td>
<td>80.67±3.71</td>
<td>430.33±3.39</td>
</tr>
<tr>
<td>04</td>
<td>CCl₄ 0.4 ml/kg</td>
<td>167.5±2.60</td>
<td>147.83±3.31</td>
<td>488.67±2.46</td>
</tr>
</tbody>
</table>

U/L = Units/Litre
5.8.3 Results:

In Group II and III, the levels of SGOT, SGPT and alkaline phosphatase were reduced significantly when compared with that of animals in carbon tetrachloride treated groups. Histopathology of liver showed corresponding arrest of necrosis with slight hydropic and fatty change (Fig. 26 and 27).

In carbon tetrachloride treated group, the levels of SGOT, SGPT and alkaline phosphatase were very high than control group and histopathological picture (Fig. 28) shows necrosis, degenerative changes (fatty and hydropic). The nuclei are pushed peripherally.

5.8.4 Discussion:

The toxins like CCl₄, which produce hepatic necrosis, are believed to do so by forming highly reactive free radicals or other unstable intermediaries in the process of metabolic degradation⁴⁹. There is a body of evidence to show that drugs which can inhibit protein synthesis can protect liver from CCl₄-induced necrosis⁵₀. The other possible mechanism is the ability of these drugs to inhibit the microsomal enzymes, thereby inhibiting or slowing down the metabolic degradation.

In our studies, CCl₄-induced necrosis and fatty changes were confirmed by histopathological studies. Significant recovery from necrosis was observed within 8 days after stopping CCl₄ in animals treated with sylimarin and ethanolic extract.
Figure-25  Photograph showing the cross section of liver exhibiting normal hepatocyte, sinusoid and portal tract.

Figure-26  Photograph showing the cross section of liver exhibiting normal hepatocyte and mild inflammation.
Figure-27 Photograph showing the cross section of liver exhibiting mild congestion and inflammation.

Figure-28 Photograph showing the cross section of liver exhibiting fatty change around portal tract, hepatocyte with fatty changes and shows peripherally pushed nuclei.
soluble part of the root of *Momordica dioica* Roxb. This was confirmed by restoration of liver enzyme (SGOT, SGPT and alkaline phosphatase) almost to control levels. Thus, hepatoprotective activity has been envisaged by the above experiment and the activity could be attributed to the saponin glycosides of triterpenoid type as reported earlier.
5.9 Anti-oxidant Activity

5.9.1 Introduction:

Reactive oxygen species (ROS) such as $O_2$, $H_2O_2$ and $OH^-$ are highly toxic to cells. Cellular antioxidant enzymes and free radical scavengers normally protect cells from toxic effects of the ROS. However, when generation of the ROS overtakes the antioxidant defence of the cells, oxidative damage of the cellular macromolecules (lipids, proteins, and nucleic acids) occurs, finally leading to various pathological conditions. ROS-mediated lipid peroxidation and DNA damage are well known outcomes of oxygen derived free radicals leading to cellular pathology and ultimately to cell death. Oxygen is vital for aerobic life processes. However, about 5% or more of the inhaled $O_2$ is converted to ROS, which however are efficiently taken care by the highly powerful antioxidant systems of the cell with minimum outward effects. When the balance between ROS production and antioxidant defences is lost, 'oxidative stress' results, which, through a series of events deregulates the cellular functions leading to various pathological conditions in almost all vital organs.

In the sequential univalent process by which $O_2$ undergoes reduction, several reactive intermediates are formed such as superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), and the extremely reactive hydroxyl radical (OH), the process can be represented as:

$$O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow OH^- \rightarrow H_2O$$
The present study was taken up in order to determine the cellular toxicity due to free radicals (oxidative stress), in the presence of various extracts of the root of *Momordica dioica* Roxb. They were evaluated for free radical scavenging ability under in *vitro* simulated conditions by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method.

5.9.2 Materials and Method:

Materials:

- Crude extracts (aqueous, ethanolic-soluble and insoluble part) of various strengths from 125 µg/ml to 4000 µg/ml.
- Ascorbic acid of various strengths from 125 µg/ml to 4000 µg/ml.
- DPPH 1 mM-3.9 mg in 10 ml ethanol.
- Vials 5 ml capacity.
- Volumetric flasks
- Micropipette–1µl
- UV-Visible spectrophotometer-Model 1601 Shimadzu make.
- Distilled water.
Different concentrations of ascorbic acid (0.5 ml) from 125 μg to 4000 μg/ml were prepared and distributed to 6 vials (5 ml) in triplicate. Same strengths of the various extracts were also prepared and distributed in triplicate (0.5 ml). To each vial, standard DPPH solution (0.5 ml) was added, mixed and kept for 20 minutes.

The ability to scavenge stable free radical DPPH was measured in UV-visible spectrophotometer at 517 nm. The absorbance of the control (water and DPPH 0.5 ml each) was also measured at 517 nm.

The results are tabulated in Table-5.17.

The % scavenging activity was calculated by using the formula

\[
\% \text{ scavenging} = \frac{\text{absorbance of standard / test} - \text{absorbance of control}}{\text{absorbance of standard / test}}
\]

Table-5.17: DPPH radical scavenging of various extracts of the root of *Momordica dioica* Roxb.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% Scavenging Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous extract</td>
</tr>
<tr>
<td>4000 μg/ml</td>
<td>71.02</td>
</tr>
<tr>
<td>2000 μg/ml</td>
<td>69.89</td>
</tr>
<tr>
<td>1000 μg/ml</td>
<td>64.47</td>
</tr>
<tr>
<td>500 μg/ml</td>
<td>38.01</td>
</tr>
<tr>
<td>250 μg/ml</td>
<td>27.47</td>
</tr>
</tbody>
</table>
5.9.3 Results:

Out of the three extracts, ethanolic extracts-soluble part showed maximum and consistent antioxidant activity (Fig. 29) in all the concentration. It was comparable with standard ascorbic acid. Other extracts also exhibited comparable and significant antioxidant activity.

5.9.4 Discussion:

Reactive oxygen species can attack vital cell components like polyunsaturated fatty acids, proteins and nucleic acids. To a lesser extent, carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage, etc., ultimately resulting in cell death. Some of the well-known consequences of generation of the free radicals in vivo are: DNA strand scission\(^2\), nucleic base modification\(^3\), protein oxidation\(^4\) and lipid peroxidation. Various extracts of the root of *Momordica dioica* Roxb. is reported to contain saponin glycosides of triterpenoid type and probably the same is responsible for the anti-oxidant activity of this plant.
Anti-oxidant activity

Figure-29

Extract (500 g/ml)

- Ascorbic acid
- Aqueous extract
- Ethanol extract-Soluble part
- Ethanol extract-Insoluble part

% Scavenging

0 10 20 30 40 50 60 70 80 90 100

87.84
38.01
65.77
48.94
References


