CHAPTER 4

Pharmacological Evaluation of Crude Extracts of the Plant
Balanites Roxburghii

Section A: Antifertility activity in female rats
Section-B: Antifertility activity in female rats
Section-C: Antimicrobial activity
Section-D: Anthelmintic activity
Section-E: Anti-inflammatory activity
Section-F: Analgesic activity
Section-G: Diuretic activity
Pharmacological evaluation of crude extracts

The plant *Balanites Roxburghii* is known to exhibit many dynamic biological and pharmacological activities\(^1\)\(^-\)\(^3\). Particularly, the fruits of this plant are being used for male antifertility by folk physicians of Chitradurga. Hence, it was contemplated to subject the crude extracts of the fruits of this plant for investigation of the following activities.

A. Antifertility in female
B. Antifertility in male
C. Antimicrobial
D. Anthelmintic
E. Anti-inflammatory
F. Analgesic
G. Diuretic

4.A Antifertility efficacy of crude extracts of the fruits of *Balanites Roxburghii* in female rats

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

1) Post-coital antifertility activity in female rats
2) Estrogenic and antiestrogenic activity in young female rats.
3) Biochemical changes.
4.A.1 Post-coital 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. Colony bred virgin female albino rats of Wistar strain were used. All animals were maintained under controlled standard animal house conditions, fed food as prescribed by Central Food Technological Research Institute (CFTRI) and water *ad libitum*. These female rats were monitored daily for different stages of estrous cycle. The rats weighing between 150-200 g of proven fertility and with regular estrous cycle were selected for this experiment. The rats, which were in proestrous stage were separated and caged with male rats that had sired litters before, in the ratio of 2:1. 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 and divided into nine groups of six animals each.

**Dose fixation**

Dose fixation was carried out by stair-case method on Swiss strain albino mice (80-100 g body weight). It was observed that none of the extracts were found to be lethal even at the dose of 3000 mg/kg body weight. Hence 1/10\(^{th}\) of 3000 mg/kg i.e. 300 mg/kg body weight of the crude extracts was fixed as the dosage. As the extracts were found to be non-toxic, double of this dose i.e. 600 mg/kg body weight was also chosen to ascertain the response of the animals and also to study the dose dependency.

The extracts were administered at the dose levels of 300 mg/kg and 600 mg/kg body weight 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.

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, weighed and examined for terratogenicity. The litters were allowed to grow to check post-natal growth and monitored for any congenital abnormalities. The observations made are presented in the Table-4.1. The photographs showing the uterus of the rat are represented in Figure-4.1 and Figure-4.2.

Table-4.1 Abortifacient activity of the extracts of fruits of *Balanites Roxburghii*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg body weight</th>
<th>Group</th>
<th>No. of rats with implantation sites on day 10</th>
<th>Mean No. of implants on day 10</th>
<th>Mean No. of litters delivered</th>
<th>Percentage of abortifacient activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>I</td>
<td>6</td>
<td>11.00</td>
<td>11.00</td>
<td>-</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>300</td>
<td>II</td>
<td>6</td>
<td>11.28</td>
<td>8.33</td>
<td>26.15</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>III</td>
<td>6</td>
<td>10.86</td>
<td>7.33</td>
<td>32.50</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>300</td>
<td>IV</td>
<td>6</td>
<td>11.63</td>
<td>9.50</td>
<td>18.31</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>V</td>
<td>6</td>
<td>11.92</td>
<td>9.16</td>
<td>23.15</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>300</td>
<td>VI</td>
<td>6</td>
<td>12.37</td>
<td>6.66</td>
<td>46.16</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>VII</td>
<td>6</td>
<td>12.94</td>
<td>5.16</td>
<td>60.12</td>
</tr>
<tr>
<td>Water extract</td>
<td>300</td>
<td>VIII</td>
<td>6</td>
<td>12.60</td>
<td>10.16</td>
<td>19.36</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>IX</td>
<td>6</td>
<td>11.62</td>
<td>9.04</td>
<td>22.20</td>
</tr>
</tbody>
</table>
Figure-4.1  Photograph of uterus of the rat without implantation sites.

Figure-4.2  Photograph of uterus showing the implantation sites.
Petroleum ether extract and ethanol extract of the plant *Balanites Roxburghii* were found to possess abortifacient activity. The phytochemical investigations have proved the presence of steroids in petroleum ether and chloroform extracts. Hence, only the ethanolic extract was taken up for the detailed investigation of estrogenic and antiestrogenic activity in Wistar strain albino rats to investigate the possible mechanism of its antifertility action.

4.A.2 Estrogenic and antiestrogenic activity

Colony bred Wistar strain immature female albino rats, aged 21-23 days, weighing between 35-45 g were bilaterally ovariectomised under light ether anaesthesia and under sterile conditions. They were divided into 4 groups consisting of 6 rats each. The first group served as control and received vehicle only (Tween-80, 1%, 5 ml/kg). The second group received ethinyl estradiol in olive oil (1 μg/rat/day) subcutaneously. The third group received ethanol extract at the dose of 600 mg/kg body weight. The fourth group received, in addition to ethinyl estradiol (1 μg/rat/day), ethanol extract at the dose of 600 mg/kg body weight. All the above treatments were given for 7 days. Vagina and the 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 were dissected out and the exact weights of the uteri were recorded. The two horns of the uterus were separated, one of the horns was used for investigation of biochemical changes and the other was utilized for histological studies. The separated horn was fixed in Bouin’s fluid for 25 hr 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. 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-4.2 and Table-4.3. The photographs of the histological observations of the uterus of the rats are represented in Figure-4.3 to Figure-4.6.

**Table-4.2** Estrogenic and antiestrogenic activity of the ethanol extract of fruits of *Balanites Roxburghii*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment group (Dose/day)</th>
<th>Uterine weight in mg ± SEM</th>
<th>Vaginal cornification</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>62.14 ± 4.52</td>
<td>nucleated and cornified cells</td>
</tr>
<tr>
<td>II</td>
<td>Ethinyl estradiol (1 μg/rat)</td>
<td>302.72 ± 13.38*</td>
<td>cornified cells</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol extract (600 mg/kg)</td>
<td>114.81 ± 9.17*</td>
<td>nucleated and cornified cells</td>
</tr>
<tr>
<td>IV</td>
<td>Ethinyl estradiol (1 μg/rat) + ethanol extract (600 mg/kg)</td>
<td>322.16 ± 9.62*</td>
<td>cornified cells</td>
</tr>
</tbody>
</table>

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

**Table-4.3** Histological changes in the uterus

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (Dose/day)</th>
<th>Diameter of the uterus (μm) ± SEM</th>
<th>Thickness of the endometrium (μm) ± SEM</th>
<th>Height of the endometrial epithelium (μm) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>356.56 ± 6.06</td>
<td>52.82 ± 2.18</td>
<td>28.14 ± 1.06</td>
</tr>
<tr>
<td>II</td>
<td>Ethinyl estradiol (1 μg/rat)</td>
<td>861.24 ± 2.84</td>
<td>247.63 ± 1.92*</td>
<td>48.72 ± 9.12**</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol extract (600 mg/kg)</td>
<td>571.40 ± 1.48**</td>
<td>75.92 ± 1.12**</td>
<td>37.63 ± 1.52*</td>
</tr>
<tr>
<td>IV</td>
<td>Ethinyl estradiol (1 μg/rat) + ethanol extract (600 mg/kg)</td>
<td>911.10 ± 2.66*</td>
<td>268.32 ± 4.38*</td>
<td>54.44 ± 3.72*</td>
</tr>
</tbody>
</table>

*p < 0.1 when compared with control, **p < 0.01 when compared with control
Figure-4.3  Photograph of the cross section of the uterus of immature control rat showing normal myometrium and very few endometrial glands.

Figure-4.4  Photograph of the cross section of the uterus of immature rat treated with ethinyl estradiol at 1 μg/rat/day showing increase in height of myometrium, endometrium and endometrial glands.

ED  Endometrium
EG  Endometrial gland
EP  Epithelium
MY  Myometrium
L   Lumen
Figure-4.5 Photograph of the cross section of the uterus of immature rat treated with ethanol extract of the fruits of *Balanites Roxburghii* at 600 mg/kg body weight showing mild estrogenic activity. Endometrial glands are visible. Height of myometrium and endometrium is increased.

Figure-4.6 Photograph of the cross section of the uterus of immature rat treated with ethanol extract of the fruits of *Balanites Roxburghii* at 600 mg/kg body weight along with ethinyl estradiol at 1 μg/rat/day showing endometrial glands, reduction in lumen and increase in thickness of endometrium.

ED    Endometrium
EG    Endometrial gland
EP    Epithelium
MY    Myometrium
L     Lumen
4.A.3 Biochemical changes

The biochemical changes in the uterus of the treated rats were carried out to know the effect of ethanol extract on the total protein content and total glycogen content of the uterus. Total protein content and glycogen content were estimated by the method as described by Lowry\textsuperscript{6} et al., and Good\textsuperscript{7} et al., respectively.

**Estimation of total protein**

Proteins are the polymers of amino acids, which are linked to each other by peptide bonds. Proteins are present in all living organisms since they form the structural and functional basis of the cell.

**Principle:** Proteins react with Folin-Ciocalteu reagent (FCR reagent) to give a blue colored complex. The color so formed is due to the reaction of alkaline copper with protein and the reduction of phosphomolybdic-phosphotungstic components in the FCR reagent by the amino acids tyrosine and tryptophan present in the protein. The intensity of blue color is measured colorimetrically.

**Procedure:** The tissue (0.5 g) was ground with ice-cold water (5 ml) and diluted with distilled water (10 ml). Aliquot sample (1 ml) was taken in a separate test tube and alkaline copper reagent (5 ml) was added, mix well and incubate at room temperature for 10 minutes. FCR reagent (0.5 ml) was added to this solution, mixed well and incubated at room temperature in dark for 30 minutes. The intensity of blue color that developed was measured colorimetrically at 660 nm.

Absorbance of solutions of standard protein at different concentrations was measured and a standard curve was constructed by plotting the concentration on x-axis and absorbance on y-axis.
Amount of protein was calculated by comparing the absorbance of tissue protein solution with the graph and using the formula

\[
\text{Amount of protein present present in sample (mg/100gm)} = \frac{\text{protein value from graph (mg)}}{\text{Aliquot sample used (1 ml)}} \times \frac{\text{Total volume of extract (ml)}}{\text{Weight of the sample (mg)}} \times 100
\]

**Estimation of glycogen**

Glycogen is the major storage polysaccharide in the liver and muscle of the animals. It is a highly branched polymer made up of D-glucose units linked by both \(\alpha-1,4\) and \(\alpha-1,6\) glycosidic bonds. In a well-fed animal, glucose is converted to glycogen in the liver, but during starvation, the liver glycogen is broken down to glucose.

**Principle:** Glycogen is released from the tissue by heating with strong alkali, precipitated by ethanol and separated by centrifugation. It is then hydrolyzed by dilute hydrochloric acid, neutralized and estimated colorimetrically.

**Procedure:** The tissue (1.5 g) was taken into a calibrated centrifuge tube containing potassium hydroxide (5N, 2 ml). It was heated on a boiling water bath for 20 minutes with occasional shaking. The resulting solution was cooled in ice, and saturated solution of sodium sulphate (0.2 ml) was added and mix thoroughly. The glycogen present was precipitated by the addition of ethanol (5 ml), cooled in ice for 5 minutes and centrifuged.

The precipitated glycogen was dissolved in water (5 ml) by gentle warming. It was diluted with water to 10 ml. Aliquot sample (1 ml) was taken into test tube and concentrated hydrochloric acid (1 ml) was added. This solution was heated on a boiling water bath for 2 hr and neutralized with sodium hydroxide using phenol red as indicator.
The neutral solution thus obtained was diluted with water (5 ml) and heated for 8 minutes after the addition of anthrone-sulphuric acid reagent (4 ml). It was cooled rapidly and the intensity of dark green color developed was measured colorimetrically at 630 nm.

Absorbance of solutions of standard glucose at different concentrations was measured and a standard curve was constructed by plotting the concentration on x-axis and absorbance on y-axis. Amount of glycogen was calculated by comparing the absorbance of tissue glycogen solution with the graph and using the formula:

\[
\text{Amount of glycogen present} = \frac{\text{Sugar value from graph (mg)}}{\text{Aliquot sample used (1 ml)}} \times \frac{\text{Total volume of extract (ml)}}{\text{Weight of the sample (mg)}} \times 100
\]

The results are tabulated in Table-4.4.

**Table-4.4** Biochemical changes in the uterus of the treated rats.

<table>
<thead>
<tr>
<th>Treatment (Dose)</th>
<th>Protein content µg/mg ± SEM</th>
<th>Glycogen content µg/mg ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.24 ± 0.40</td>
<td>1.52 ± 0.20</td>
</tr>
<tr>
<td>Ethinyl estradiol (1 µg/kg)</td>
<td>10.92 ± 0.84</td>
<td>1.92 ± 0.45</td>
</tr>
<tr>
<td>Ethanol extract (600 mg/kg)</td>
<td>8.78 ± 0.30</td>
<td>1.50 ± 0.11</td>
</tr>
<tr>
<td>Ethinyl estradiol (1 µg/kg) + ethanol extract (600 mg/kg)</td>
<td>12.41 ± 0.15</td>
<td>2.06 ± 0.5</td>
</tr>
</tbody>
</table>
Results

All the extracts were evaluated for post-coital antifertility activity at the dose of 300 mg/kg and 600 mg/kg body weight. No toxic effects were observed either by gross visual examination or in the weight of rats. After discontinuation of the treatment, all the rats were mated. This resulted in pregnancy and delivery of normal litters, indicating that the action of the extracts of fruits of *Balanites Roxburghii* is reversible.

There was no noticeable change in the color and size of the implants when compared with rats of control group. These observations clearly indicate that none of the extracts possess antiimplantation activity. However, all the extracts exhibited abortifacient activity. Amongst these, ethanol extract showed 46.16% abortifacient activity at a dose of 300 mg/kg body weight and 60.12% at a dose of 600 mg/kg body weight. All the animals in treated group showed open vagina, while the control group had closed vagina. No vaginal bleeding was observed in any of the animals. This observation revealed that all the implants have been absorbed by the uterus.

Oral administration of the ethanol extract caused significant increase in uterine weight. The uterotropin potency, as shown by the increase in weight of the uterus, was about 40% in the case of ethanol extract, when compared with that of ethinyl estradiol. This suggested mild estrogenic activity of the extract. 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 also observed that the ethanol extract 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.

Examination of the vaginal smears of the treated rats showed predominantly cornified and nucleated epithelial cells.

The results of biochemical changes also support the observations made in histological studies, that the ethanol extract of *Balanites Roxburghii* has mild estrogenic activity. Administration of ethanol extract along with ethinyl estradiol at the dose of 600 mg/kg body weight and 1 μg/rat/day respectively for seven days, resulted in increase in protein as well as glycogen content in the uterus.

**Discussion**

In the present study, the extracts of the fruits of *Balanites Roxburghii* were tested at the dose of 300 mg/kg and 600 mg/kg body weight. All these extracts did not exhibit any antiimplantation activity. However, abortifacient activity was exhibited by these extracts. The ethanol extract showed 46.16% abortifacient activity at a dose of 300 mg/kg body weight and 60.12% at a dose of 600 mg/kg body weight.

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 levels of these hormones can cause antiimplantation 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 unfavorable 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.
4.B Antifertility efficacy of the ethanol extract of the fruits of *Balanites Roxburghii* in males.

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 the discovery of this, many researchers have taken up screening of various plants and their part/s for evaluation of antifertility effect in males.

Rural folk physicians of Chitradurga use the fruits of *Balanites Roxburghii* for antifertility 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 and antiandrogenic effect of the ethanol extract of fruits of *Balanites Roxburghii*.

The antifertility activity of the ethanol extract of fruits of *Balanites Roxburghii* in young male rats was ascertained by studying the following parameters.

1) Androgenic/antiandrogenic activity in young male rats.
2) Biochemical changes.

4.B.1 Androgenic/antiandrogenic activity

The androgenic/antiandrogenic activity has been carried out by following the method as described by Lohiya and Goyal.

Materials and Method

Colony bred young male albino rats, (Wistar strain) 22 days old and weighing between 28-32 g, were used for the 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%, 5 ml/kg). The second
group received testosterone suspension in 1% Tween-80 solution at the dose of 1 mg/kg subcutaneously. The third group received the suspension of ethanol extract in Tween-80 (0.1%, 1 ml) at 600 mg/kg body weight. The fourth group received, in addition to testosterone, a test dose of the ethanol extract at 600 mg/kg body weight. 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 the ventral prostrate were dissected out, freed from surrounding tissues and weighed quickly on a sensitive balance. Gravimetric changes were recorded and shown in Table-4.5 and Table-4.6. The photographs showing the histological observations of the testes of the rats are represented in Figure-4.7 to Figure-4.10.

**Table-4.5** Effect of the ethanol extract of the fruits of *Balanites Roxburghii* on the weights of male reproductive organs when fed orally to young male rats for seven days (in mg/100 g body weight ± SEM).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment dose (mg/kg)</th>
<th>Testes ± SEM</th>
<th>Seminal vesicle ± SEM</th>
<th>Ventral prostrate ± SEM</th>
<th>Vas deferens ± SEM</th>
<th>Epididymes ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>647.76 ± 16.26</td>
<td>49.26 ± 2.16</td>
<td>56.68 ± 6.21</td>
<td>31.52 ± 3.65</td>
<td>79.42 ± 6.84</td>
</tr>
<tr>
<td>II</td>
<td>Testosterone (1 mg/kg)</td>
<td>654.32 ± 14.62*</td>
<td>78.45 ± 0.52*</td>
<td>89.46 ± 4.83**</td>
<td>44.81 ± 4.58*</td>
<td>88.73 ± 8.14*</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol extract (600 mg/kg)</td>
<td>586.31 ± 31.45*</td>
<td>36.73 ± 1.68**</td>
<td>42.84 ± 4.29+</td>
<td>26.07 ± 3.12*</td>
<td>61.03 ± 7.65+</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanol extract (600 mg/kg) + testosterone (1 mg/rat)</td>
<td>642.05 ± 24.31***</td>
<td>38.31 ± 1.74*</td>
<td>45.97 ± 3.12**</td>
<td>29.76 ± 2.28**</td>
<td>62.82 ± 4.45*</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
Figure-4.7  Photograph showing the cross section of testes of immature control male rat.

Figure-4.8  Photograph showing the cross section of testes of immature male rat treated with testosterone at 1 mg/rat/day.
Figure-4.9  Photograph showing the cross section of testes of immature male rat treated with ethanol extract of the fruits of *Balanites Roxburghii* at 600 mg/kg body weight.

Figure-4.10  Photograph showing the cross section of testes of immature male rat treated with ethanol extract of the fruits of *Balanites Roxburghii* at 600 mg/kg body weight along with testosterone at 1 mg/rat/day.
Table-4.6 Changes in the testes due to oral administration of the extracts of *Balanites Roxburghii* on young male rats for seven days.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (Dose)</th>
<th>Diameter of the testes (μm) ± SEM</th>
<th>Diameter of the seminiferous tubules (μm) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>4132 ± 23.7</td>
<td>132.7 ± 4.1</td>
</tr>
<tr>
<td>II</td>
<td>Testosterone (1 mg/kg)</td>
<td>4145 ± 16.2*</td>
<td>145.8 ± 2.9**</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol extract (600 mg/kg)</td>
<td>3986 ± 08.7**</td>
<td>124.4 ± 3.6***</td>
</tr>
<tr>
<td>IV</td>
<td>Testosterone (1 mg/kg) + Ethanol extract (600 mg/kg)</td>
<td>4016 ± 12.3*</td>
<td>125.8 ± 3.1**</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.

4.2.2 Biochemical changes

The biochemical changes of the testes were also carried out by the methods as described earlier. The results are recorded in Table-4.7.

Table-4.7 Biochemical changes in the testes of the treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein content μg/mg ± SEM</th>
<th>Glycogen content μg/mg ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.16 ± 0.2</td>
<td>2.24 ± 0.4</td>
</tr>
<tr>
<td>Testosterone (1 mg/kg)</td>
<td>5.40 ± 0.5</td>
<td>1.23 ± 0.6</td>
</tr>
<tr>
<td>Ethanol extract (600 mg/kg)</td>
<td>6.22 ± 0.3</td>
<td>1.44 ± 0.1</td>
</tr>
<tr>
<td>Testosterone (1 mg/kg) + ethanol extract (600 mg/kg)</td>
<td>6.53 ± 0.6</td>
<td>1.64 ± 0.5</td>
</tr>
</tbody>
</table>
Results

The administration of ethanol extract of the fruits of *Balanites Roxburghii* to prepubertal male rats for seven days has significantly reduced the weights of testes, epididymes, seminal vesicle and ventral prostrate when compared with the rats of control and testosterone group. A significant increase in the weights of testes and other accessory organs was observed when testosterone was administered alone, but androgenic effect of testosterone was decreased with simultaneous administration of the ethanol extract and testosterone, which was indicated by the 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.

The diameter of the testes and seminiferous tubules decreased significantly in ethanol extract treated rats, when compared with the control rats and testosterone treated rats. The seminiferous tubules of the control rats contained healthy spermatogonia, both primary and secondary spermatocytes, and a few spermatids indicating the normal spermatogenesis. However the seminiferous tubules of ethanol extract treated rats showed less spermatocytes and a very few spermatids.

Decreased levels of glycogen and protein content proved the catabolic nature i.e. antiandrogenic effect of the ethanol extract. The inhibition of spermatogenesis may also be due to low availability of glycogen, which is a nutrient for spermatogenesis.
4.C Antimicrobial activity

It is a well known fact that the interactions between microorganisms, plants and animals are natural and constant. The ecological role of microorganisms and their importance in the bio-geochemical cycles in nature is well documented. The human food supply consists basically of plants and animals or products derived from them.

Microorganisms are ubiquitous and are so small that they can only be visualized with the aid of high-resolution microscopes.

Microbes are a heterogeneous group of several classes of living things. These were originally classified under the plant and animal kingdoms. As this proved unsatisfactory, a five-kingdom system was introduced namely,

a. Monera (Unicellular prokaryotes)
b. Protista (Unicellular Eukaryotes)
c. Fungi
d. Plantae
e. Animalia

Protozoa are unicellular, non-photosynthetic eukaryotic microorganisms. Various groups of protozoa exhibit differing strategies of locomotion, for example Ameoba can change its cell shape by extending the cell wall and thus migrates along, whereas Paramecium is propelled by numerous beating structures called cilia.

As group, fungi are microorganisms that obtain their nutrition from organic compounds. Their cells are usually surrounded by protective cell wall composed of chitin or other polysaccharides.
Fungi produce spores, which are specialized cells, involved in reproduction, dissemination and survival. Depending on the cell morphology fungi can be divided into four classes such as, yeast, yeast like fungi, moulds and dimorphic fungi.

Yeasts are unicellular and other fungi called moulds are filamentous. Dimorphic fungi may grow as mycelial or yeast-form depending upon prevalent growth conditions. Fungi form multicellular filaments called hyphae. The only pathogenic yeast is *Cryptococcus neoformans*. Fungi have been recognized as causative agents of human disease earlier than bacteria. Fungal infections are extremely common and some of them are serious and even fatal.

Antimicrobial activity of the crude extracts of the fruits of *Balanites Roxburghii* was carried out in two parts namely, antibacterial and antifungal activities.
**4.C.1 Antibacterial activity**

**Introduction**

The antibacterial activity of the extracts of *Balanites roxburghii* was studied comparatively with that of standard ciprofloxacin, by cup-plate method using a gram-positive and a gram-negative organisms *Staphylococcus aureus* and *Klebsiella pneumoniae*.

**Materials and method**

1. Nutrient agar
2. Sterilized petridishes, pipettes, boiling tubes and beakers
3. Sterilized test tubes
4. Sterile 6 mm borer
5. Sterile inoculation loops
6. Sterilized fine pointed forceps
7. Tuberculin syringes

**Preparation of media**

Nutrient agar was prepared by dissolving of nutrient agar (14 g) in distilled water (500 ml), the pH of the solution was adjusted to 7.4 and then sterilized for 15 minutes at 15 lbs pressure in autoclave.

**Preparation of sub-cultures**

One day prior to the experiment, the microorganisms were inoculated into sterilized nutrient broth tubes and incubated at 37°C for 24 hr. The organisms used in the present study for evaluating antibacterial activity of test compounds were obtained from the laboratory at National College of Pharmacy, Shimoga. On the day of testing, the
organisms were subcultured into sterile nutrient broth. After incubating the same for three hours, the growth thus obtained was used as inoculum for the test.

**Sterilization of media and glassware**

The media used in the present study, nutrient agar and nutrient broth, were sterilized in conical flasks of suitable capacity by autoclaving at 15 lbs pressure for about 20 minutes. The cork borer, petridishes, test tubes and pipettes were sterilized in hot air oven at 160°C for an hour.

**Preparation of test solutions of the extracts**

The suspension of each extract (500 mg) in Tween-80 (0.1%, 1 ml) was dissolved in distilled water (10 ml) in suitably labeled sterile test tubes separately, to get the solution of the extract of 50 mg/ml concentration.

**Method of testing : Cup-Plate Method**

This method depends on the diffusion of an antibiotic from a cavity through the solidified agar layer in a petridish to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around the cavity containing a solution of antibiotic.

A previously liquified medium was inoculated appropriate to the assay with the requisite quantity of the suspension of the microorganisms between 40-50°C and the inoculated medium was poured into petridishes to give a depth of 3 to 4 mm., ensuring that the layers of medium were uniform in thickness by placing the dishes on a leveled surface.
The dishes thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes were used and the surface or the agar layer was dry at the time of use. With the help of a sterile cork borer, three cups of each 6 mm diameter were punched and scooped out the set agar in each petridish (three cups were numbered for the particular compounds, solvent and a standard) using sterile pipettes, the standard and the sample (0.1 ml) of known concentration were fed into the bored cups. The dishes were left standing for 2 hr. at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time among the application of different solutions. These were then incubated for 24 hr at 37°C. The zone of inhibition developed, if any, was then accurately measured and recorded. Each zone of inhibition recorded was average of three measurements. Zone of inhibition for Tween-80 was done separately.

The data of antibacterial activity of standard and the extracts is given in Table-4.8.
4.C.2 Antifungal activity

Introduction

The antifungal activity of the extracts of *Balanites Roxburghii* was studied in comparison with that of standard antifungal drug, ciclopirox olamine, by cup-plate method.

Materials and method

1. Potato dextrose agar
2. Micropipette
3. Sterilized petridishes
4. Potato dextrose broth (48 hours old)
5. Sterile test tubes for preparation of solutions of the test compounds.

Sterilization of media and glassware

The media used in the present study, nutrient agar and nutrient broth, were sterilized in conical flasks of suitable capacity by autoclaving at 15 lbs pressure for about 20 minutes. The cork borer, petridishes, test tubes and pipettes were sterilized in hot air oven at 160°C for an hour.

Preparation of test solutions of the extracts

The suspension of each extract (500 mg) in Tween-80 (0.1%, 1 ml) was dissolved in distilled water (10 ml) in suitably labeled sterile test tubes separately, to get the solution of the extract of 50 mg/ml concentration.
**Preparation of media**

Potato dextrose agar was prepared by dissolving potato dextrose (20 g) agar in distilled water (500 ml), the pH of the solution was adjusted to 5.6 and then sterilized for 15 minutes at 121°C at 15 lbs pressure in an autoclave.

**Preparation of sub-culture**

Two-days prior to the experiment, the microorganism were inoculated into sterilized potato dextrose broth tubes and incubated at 25°C for 48 hr.

**Method of testing: Cup-plate method**

This method depends on the diffusion of an antifungal agent from a cavity through the solidified agar layer in a petridish to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around the cavity containing a solution of antifungal agent.

A previously liquified medium was inoculated appropriate to the assay with the requisite quantity of the suspension of the microorganisms between 40-50°C and the inoculated medium was poured into petridishes to give a depth of 3 to 4 mm., ensuring that the layers of medium were uniform in thickness by placing the dishes on a leveled surface.

The dish thus prepared were stored in a manner so as to ensure that no significant growth of death of the test organism occurs before the dishes were used and the surface or the agar layer was dry at the time of use. With the help of a sterile cork borer, three cups of each 6 mm diameter were punched and scooped out the set agar in each petridish using sterile pipettes, the standard and the sample solution (0.1 ml) of known concentrations were fed into the bored cups.
The dishes were left standing for 2 hr at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time among the application of different solutions. These were then incubated for three days at 25°C. The zone of inhibition developed, if any, was then accurately measured and recorded in the tables. Each zone of inhibition recorded was average of three measurements.

The data of antifungal activity of standard and the extracts is given in Table-4.8.

Table-4.8 Antimicrobial activities of the extracts of Balanites Roxburghii.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antibacterial activity Zone of inhibition (in mm.)</th>
<th>Antifungal activity zone of inhibition 'in mm.'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>K. pneumoniae</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>08</td>
<td>13</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>07</td>
<td>09</td>
</tr>
<tr>
<td>Water extract</td>
<td>06</td>
<td>08</td>
</tr>
</tbody>
</table>

Results

The results revealed that both petroleum ether extract and chloroform extract exhibited antimicrobial activity. Considerable zone of inhibition was observed for chloroform extract against K. pneumoniae when compared to the standard drug. The chloroform extract has also shown good antifungal activity against C. albicans and A. niger. The antimicrobial activity of other extracts was less when compared with the standard.
4.D Anthelmintic Activity

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 helminthius infection can be acquired by,

1. Contact with infected animal
2. The ingestion of infected meat
3. The animal or human excreta via ground water
4. 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 helminthiasis 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 paralyzef 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*
(Tape worm), *Schistosomes* (Trematodes, flukes), *Filaria* (Thread worm), *Nematodes*
(Round worm) and *Pheritima posthuma* (Earthworms, order-annelida, class-Oligichaeta).

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 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 fruits of *Balanites Roxburghii* are reported to exhibit anthelmintic activity
and henceforth, it was contemplated to investigate the various extracts of the fruits for
possible anthelmintic activity. As it was difficult to procure either roundworm or
tapeworm, the experiment was performed on earthworms, which have similar life cycle.

The technique adopted was that described by Giand et al., with slight
modification.
Anthelmintic activity was evaluated by using earthworm *Pheritima posthuma* (class-Annélida and order-Oligicheata).

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, 2 ml of each extract (50 mg/ml) in 0.1% Tween-80 suspension were placed and the volume was made up to 25 ml with an aqueous solution of dextrose (6%). In another pretidish Tween-80 (25 ml, 0.1% solution) prepared in 6% dextrose solution was placed, which served as control. 1 ml of piperazine citrate (100 mg/ml) suspended in Tween-80 (0.1%, 1 ml) was placed in another petridish and the volume was made up to 25 ml using dextrose (6%) solution. The piperazine citrate solution was served as standard. In each pretidish 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. The results were tabulated in Table-4.9. The Figure 4.11 shows the worms in 1% Tween-80 solution and Figure-4.12 shows the worms in the solution of chloroform extract of the fruits of *Balanites Roxburghii* in dextrose solution.
Figure-4.11  Photograph showing earthworms in 1% Tween-80 solution.

Figure-4.12  Photograph showing earthworms in the solution of chloroform extract of the fruits of *Balanites Roxburghii*. 
Table-4.9 Anthelmintic activity of the extracts of *Balanites Roxburghii*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of earthworms tested</th>
<th>Concentration (Wt./Vol., mg)</th>
<th>Mean paralysis time (in min.)</th>
<th>Mean death time (in min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>-</td>
<td>NP</td>
<td>ND</td>
</tr>
<tr>
<td>Standard</td>
<td>8</td>
<td>100</td>
<td>120</td>
<td>160</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>8</td>
<td>100</td>
<td>142</td>
<td>200</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>8</td>
<td>100</td>
<td>130</td>
<td>190</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>8</td>
<td>100</td>
<td>162</td>
<td>ND</td>
</tr>
<tr>
<td>Water extract</td>
<td>8</td>
<td>100</td>
<td>NP</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = no death NP = no paralysis

**Results**

The chloroform extract and petroleum ether extracts of the fruits of *Balanites Roxburghii* exhibited significant anthelmintic activity against earthworm at 100 mg/ml concentration when compared with the standard piperazine citrate. However ethanol extract and water extract showed very less anthelmintic activity against earthworms.
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 paralyze 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 similar type of action.
4.E Anti-inflammatory Activity

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, 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, 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.

An attempt was made to test the anti-inflammatory properties of the extracts of the fruits of *Balanites Roxburghii*.

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 inflammagens. Substances such as carrageenan, formalin, bradykinin, histamine, 5-hydroxytryptamine 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.

**Materials and Method**

Albino Rats weighing 150-200 g of Wistar strain

Plethysmograph

Beaker and measuring jar.

Standard drug (Ibuprofen).

Potassium permanganate solution (0.1%).

Tween-80 solution (0.1%).

Carrageenan (0.1%).
Rat paw edema method

This method was based on plethysmographic measurement of carrageenan-induced acute rat paw edema\textsuperscript{16-20} produced by subplantar injection of carrageenan in hind paw of the rat. The method described by Wilhlimi and Domenjoz\textsuperscript{21} later on modified by Sirodia and Rao\textsuperscript{22} was used for measuring the paw volume. The photographs showing plethysmograph and the measurement of rat paw volume\textsuperscript{23-27} are given in Figure-4.13 to Figure-4.16.

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 stopcock ‘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
Figure-4.13  Photograph showing the carrageenan being injected in the right hind paw for inducing edema.

Figure-4.14  Photograph showing the inflamed right hind paw.
Figure-4.15  Photograph showing plethysmograph used for measuring paw volume.

Figure-4.16  Photograph showing the inflamed right hind paw of the rat being dipped in plethysmograph for measuring the paw volume.
bubble in limbs ‘A’ and ‘B’, was kept closed through out 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 6 groups of 4 animals each. The group I served as control and received Tween-80 (0.1%, 1 ml) solution orally. The group II received Ibuprofen at a dose of 40 mg/kg body weight in Tween-80 (0.1%, 1 ml) and served as standard. The group III, IV, V and VI received orally the petroleum ether extract, chloroform extract, ethanol extract and water extract respectively at the dose of 600 mg/kg body weight in Tween-80 (0.1%, 1 ml) solution. These drugs were administered one hour before the injection of the irritant. After 1 hr all the animals were injected subcutaneously carrageenan solution (0.1%, 0.05 ml) to the right hind paw in the subplantar region, immediately the paw volume was measured (‘0’ hour). After 3 hr the paw volume was measured in control, in standard and in test animals.

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

\[ \frac{1}{V_c} \times 100 \]

Where, \( V_t \) = Mean increase in the paw volume in test animal group.

\( V_c \) = Mean increase in the paw volume in control group.

Statistical analysis was carried to determine % protection,
### Table-4.10 Anti-inflammatory activity of the extracts of *Balanites Roxburghii*

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Difference in paw volume ± SEM after 3 hr</th>
<th>Percentage inhibition of edema after 3 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>0.600 ± 0.028</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Ibuprofen</td>
<td>40</td>
<td>0.146 ± 0.046</td>
<td>75.7</td>
</tr>
<tr>
<td>III</td>
<td>Petroleum ether extract</td>
<td>600</td>
<td>0.441 ± 0.078</td>
<td>26.4</td>
</tr>
<tr>
<td>IV</td>
<td>Chloroform extract</td>
<td>600</td>
<td>0.372 ± 0.061</td>
<td>38.0</td>
</tr>
<tr>
<td>V</td>
<td>Ethanol extract</td>
<td>600</td>
<td>0.320 ± 0.420</td>
<td>46.7</td>
</tr>
<tr>
<td>VI</td>
<td>Water extract</td>
<td>600</td>
<td>0.403 ± 0.325</td>
<td>32.8</td>
</tr>
</tbody>
</table>

Index for anti-inflammatory activity.

- **Model**: Acute inflammation
- **Method**: Carrageenan induced edema
- **Animals**: Albino rats
- **Number of animals per group**: 4
- **Route of administration**: Oral
- **Standard**: Ibuprofen
- **Control**: Tween-80 (0.1%)
- **S.E.M.**: Standard Error Mean.
Results

The four extracts of the fruits of *Balanites Roxburghii* were tested for anti-inflammatory activity against carrageenan–induced rat paw edema. The activity was compared with Ibuprofen. Ethanol extract and water extract showed considerable anti-inflammatory activity when compared with the standard drug.

Discussion

The results clearly indicate that ethanol extract and water extract 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 the 1 hr after administration of carrageenan, a more pronounced phase, 2nd 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 requires more investigation.
4.F Analgesic activity

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 form herbal extracts were used by earlier physicians for this purpose. Alcohol and methanolic 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 Serturner, 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.

1. Structural modification of morphine
2. Synthesis of morphine like compounds
3. Synthesis of other heterocyclic compounds
4. Random screening of synthesized compounds
5. Subjecting different plant extracts for analgesic response on mice or rats
6. Isolation of active components from plant extracts

It is a well known fact that plant possessing anti-inflammatory property can act as analgesic also. As various extracts of the fruits of the Balanites Roxburghii have been shown to possess significant anti-inflammatory activity, it is envisaged now to subject various extracts for analgesic activity in albino mice.
There are mainly three methods available to evaluate analgesic activity namely,

a. Acetic acid induced writhing method in mice

b. Tail flick method in rats

c. Eddy's hot plate analgesiometer method in mice

In the present investigation, analgesic activity of various extracts was evaluated by using acetic acid induced writhing method in mice.

**Acetic acid induced writhing method**

The analgesic activity of the extracts of *Balanites Roxburghii* was done on albino mice.

**Materials and Method**

Albino mice weighing 22-35 g of Swiss strain.

Acetic acid (0.6%).

Standard drug (Acetyl salicylic acid).

Stop clock.

Tween-80 solution (0.1%).

Colony bred albino mice (Swiss strain) of either sex weighing 22-35 gm were used to evaluate analgesic activity. Analgesic activity was determined as described by the method based on acetic acid induced writhing in mice. To study the response of various extracts of *Balanites Roxburghii* for analgesic activity, 24 mice were selected and divided into 6 groups comprising of 4 animals each. All the animals received 0.6% acetic acid solution (10 ml/kg body weight) intraperitoneally and number of writhing were recorded after 5 minutes for a period of 20 minutes. 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 of Tween-80 (0.1%, 0.5 ml) and served as control, second group received the suspension of acetyl salicylic acid (150 mg/kg body weight) in Tween-80 (0.1%, 5 ml) orally and served as standard. The animals in III, IV, V and VI groups received various extracts of the fruits of *Balanites Roxburghii* at a dose of 200 mg/kg suspended in Tween-80 (0.1%). After one hour, 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 the duration of 20 min. The results are recorded in the Table-4.11. The photographs showing analgesic activity are represented in Figure-4.17 and Figure-4.18.

The percentage protection was calculated by using the formula

\[
\text{Percentage protection} = 1 - \frac{V_t}{V_c} \times 100
\]

Where, \(V_t\) = Mean number of writhing in test animals
\n\[V_c\] = Mean number of writhing in control.

| Table-4.11 Analgesic activity of the extracts of *Balanites Roxburghii*. |
|-----------------------------|-------------------|-----------------------------|-----------------------------|-----------------------------|
| **Group** | **Compound** | **Dose (mg/kg)** | **Mean No. of writhing** | **Mean No. of writhing** | **Percentage protection** |
| | | | Without the administration of the drug | With the administration of the drug | |
| | | | ± SEM | ± SEM | |
| I | Control | - | 45.83±1.28 | 45.83±1.28 | - |
| II | Acetyl salicylic acid | 150 | 47.00±2.50 | 11.80±1.27 | 74.9 |
| III | Petroleum ether extract | 600 | 20.50±1.58 | 07.83±1.79 | 61.8 |
| IV | Chloroform extract | 600 | 40.00±2.73 | 14.33±3.88 | 64.2 |
| V | Ethanol extract | 600 | 28.76±2.17 | 12.18±1.06 | 57.6 |
| VI | Water extract | 600 | 27.34±1.56 | 17.67±1.89 | 35.4 |
**Figure-4.17**  Photograph showing the mice being injected with acetic acid for inducing writhing.

**Figure-4.18**  Photograph showing the mice exhibiting writhing.
Index for analgesic activity.

Method : Acetic acid induced writhing
Animals : Swiss albino mice
Number of animals per group : 4
Route of administration : Oral
Standard : Acetyl salicylic acid
Control : Tween-80 (0.1%)
S.E.M. : Standard Error Mean.

Results

The observations revealed that the petroleum ether, chloroform and ethanolic extract exhibited considerable analgesic activity when compared with that of standard drug and water extract showed poor analgesic effect. Among all the extracts, the chloroform extract was found to exhibit maximum analgesic activity.

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. The petroleum ether, chloroform and ethanolic extracts of the fruits of *Balanites Roxburghii* exhibited significant analgesic activity when compared with acetyl salicylic acid.
4.G Diuretic activity

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 the extracellular fluid volume by decreasing the total sodium chloride content of the body. A sustained imbalance between the dietary sodium loss is incompatible with the 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.

Materials and Method

Albino rats weighing 150-200 g of (Wistar strain).

Tween-80 solution (0.1%).

Stop clock.

Standard drug (Frusemide).

Metabolic cage.

Measuring jar.
For evaluation of diuretic activity on albino rats (Wistar strain) the method adopted by Lipschitz\(^9\) was employed. Rats of either sex, weighing between 100-200 g were divided into 6 groups each containing 4 animals. Group one served as control and received 5 ml of 0.1% Tween-80 in distilled water orally. Group two received 40 mg/kg body weight of frusemide in 0.1% Tween-80 orally and served as standard. Group 3, 4, 5 and 6 received the extracts at the dose of 600 mg/kg body weight suspended in 0.1% Tween-80 orally. Each group of animals was kept in different metabolic cages provided with a wire mesh at the bottom and a funnel to collect urine. Sieves made up of stainless steel were placed on the funnel to retain feces. The rats were fed with standard diet and water \textit{ad libitum}. Food and water were withdrawn 24 hr prior to the experiment. Urine excretion was collected after 5 hr and the values were tabulated in Table-4.12. The Figure-4.19 shows the rats in metabolic cage.

Table-4.12 Diuretic activity of the extracts of \textit{Balanites Roxburghii}.

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Volume of urine collected after 5 hr.</th>
<th>T/S (Lipschitz values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>600</td>
<td>08</td>
<td>0.27</td>
</tr>
<tr>
<td>II</td>
<td>Standard</td>
<td>600</td>
<td>29</td>
<td>1.00</td>
</tr>
<tr>
<td>III</td>
<td>Petroleum ether extract</td>
<td>600</td>
<td>14</td>
<td>0.55</td>
</tr>
<tr>
<td>IV</td>
<td>Chloroform extract</td>
<td>600</td>
<td>17</td>
<td>0.58</td>
</tr>
<tr>
<td>V</td>
<td>Ethanol extract</td>
<td>600</td>
<td>12</td>
<td>0.41</td>
</tr>
<tr>
<td>VI</td>
<td>Water extract</td>
<td>600</td>
<td>8</td>
<td>0.69</td>
</tr>
</tbody>
</table>
Figure-4.19  Photograph showing rats in metabolic cage.
Index for diuretic activity.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Albino rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals per group</td>
<td>4</td>
</tr>
<tr>
<td>Standard</td>
<td>Frusemide</td>
</tr>
<tr>
<td>T</td>
<td>Urine collected for extracts</td>
</tr>
<tr>
<td>S</td>
<td>Urine collected for standard drug</td>
</tr>
<tr>
<td>Control</td>
<td>Tween-80 (0.1%)</td>
</tr>
</tbody>
</table>

**Results**

The Lipschitz value for the standard drug Frusemide is taken as 1. The tested extracts of *Balanites Roxburghii* showed less T/S value. Hence, only water extract exhibited diuretic property and the other extracts showed less diuretic activity.
References


