ANTIFERTILITY EFFICACY OF 50% ETHANOLIC EXTRACT OF CALENDULA OFFICINALIS IN MALE RATS

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Received: 6 July 2011, Revised and Accepted: 2 Nov 2011

ABSTRACT

The reproductive effect of the ethanolic extract of Calendula officinalis was evaluated by oral administration at the dose level of 150, 250 and 500 mg/rat/day for 60 days. After 55 days of treatment, male rats were kept for mating with female rats. At termination on 61st day the rats were sacrificed & the blood was collected by cardiac puncture for hematological & serological studies. The reproductive organs (testes, epididymis, vas deferens, seminal vesicle and prostate gland) were dissected out and processed for biochemical estimation. The body weights were not affected whereas the weights of reproductive organs viz. testes, epididymis, seminal vesicles, vas deferens and prostate gland were decreased significantly (P<0.01). The value of glycogen, protein, sialic acid and fructose of reproductive organs were also decline significantly (P<0.01). The level of cholesterol and lipid per oxidation were significantly increased however glutathione in testes showed low values. Serum testosterone was decline after the treatment. Hematological profiles were remained in normal range. The sperm motility and density of cauda epididymis were declined highly significantly (P≤0.001). It is concluded that oral administration of 50% ethanolic extract of Calendula (Plant) showed a significant effect on fertility in male rats without interfering general physiology of rats.

Keywords: Reproductive organs, Lipid per oxidation, Glutathione, Testosterone

INTRODUCTION

The population of India is multiplying at alarming rate. The control of human fertility in the sense of its limitation is the most important and urgent of all biosocial and medical problem confronting mankind today. During the recent past decades a large number of plant species mentioned in old Material, Medica and Ayurvedic literature have been screened and searched thoroughly for their antifertility effect on males.

Calendula officinalis Linn. (Asteraceae) commonly known as Pot Marigold, is an important medicinal plant. C. officinalis contains flavonols, glycosides, triterpene, oligoglycosides, triterpene glycosides, saponine and sesquiterpenes glycosides. C. officinalis is phytotherapeutic plant rich in biologicallyactive metabolites like sesquiterpenes, alcohol, triterpene, flavonoids, hydroxycumarin, carotenoids, tannins and volatile oils (0.1 -0.2%). It is phytoterapic plant rich in biologically active compounds which have proved to be useful against large number of diseases.

MATERIAL AND METHOD

Plant Extract

Calendula officinalis (plant) was collected from Nursery, University of Rajasthan Jaipur. Botanical identification was authenticated at the Department of Botany, University of Rajasthan in comparison with the pre existing voucher specimen (RUBL 20541). The plant was shade dried and powered. 100 gm. powder was subjected to COEtEx daily for 60 days.

Group I - Vehicle (Distilled water) 0.5 ml/day/rat.
Group II - Animal received (COEtEx) 150 mg/kg b.wt/day/rat.
Group III - Animal received (COEtEx) 250mg/ kg b.wt/day/rat.
Group IV - Animal received (COEtEx) 500mg/ kg b.wt/day/rat.

Fertility Test

The mating exposure test of control and treated group were performed on day 50-60 using the method of W.H.O. The mated females were separated and the implantation sites and number of foetuses were recorded on 21st day of pregnancy through laprotony.

Autopsy

The rats were weighed and autopsied under light ether anesthesia, 24 hour after last dose of treatment. The blood from heart was collected in pre heparinized tubes for hematological studies and serum was separated for serological studies.

Body and organ weight measurements

Initial and final body weights of the animals were recorded. At autopsy, the reproductive organs (Testes, Epididymis, Seminal Vesicle, Prostate, Vas deferens) and vital organs (Adrenal, Heart, Liver, Kidney,) were taken out trimmed free of fat and weighed separately on electronic balance to the nearest milligram.

Sperm motility and density

After anesthetizing the rats, the epididymis was exposed by scrotal incision and spermatozoa were expressed out by cutting the distal end of the cauda epididymal tubules.

Spermatozoa with epididymal fluid was diluted with physiological saline and placed on a Neubauer’s chamber slide and motility rate (rate
and percentage) of 100 spermatozoa per rat was observed under
microscope using pre calibrated ocular micrometer 17.
Spermatozoa were counted by placing the sperm suspension on both
slides of Neubauer’s hemocytometer and allowed to settle in a
humid chamber for one hour. The number of spermatozoa in the
appropriate squares of Neubaure’s camber slide was calculated
under the microscope at 100 X magnification 18.

Blood and Serum analysis
Blood was collected in the heparinized tubes by cardiac puncture
and the value of R.B.C. and W.B.C counts 19. Hematocrit and
Hemoglobin were estimated. The value of M.C.V., M.C.H., and M.C.H.
were also calculated from the value of R.B.C. counts, Hematocrit, and,
Hemoglobin %. The serum was separated to estimate the SGOT,
SGPT, Alkaline phosphatases and Acid phosphatase according to the
standard methods.

Tissue Biochemistry
Biochemical estimation of fresh frozen tissues were done by
Colorimeter. The tissues were analyzed for the various parameters.
Total protein in testes, epididymis, seminal vesicle and prostate 20,
Glycogen in testes and liver 21. Fructose in seminal vesicle 22 Total
cholesterol in testes, and liver 23 Lipid peroxidation in testes 24,
Glutathione (GSH) in testes 25.

Hormones
Estimation of testosterone, FSH (Follicle stimulating hormone) and
LH (Luteinizing hormone) were performed with the help of ELISA

Statistical Analysis
All of the recorded values of body/organ weight, sperm dynamics,
hematological parameters and testicular cell dynamics were
expressed in terms of mean ± SEM. The treated groups will be
compared to control using the students’ test.

RESULTS
Oral administration of Calendula officinalis at all the dose level did
not cause any significant change in the body weight of rats (Table 1).
However COEtEx caused a highly significant decreased in the weight
of testes, epididymis, seminal vesicles, ventral prostate and vas
deferens in dose dependent manner (Table 1). Feeding of COEtEx to
male rats caused significant to highly significant drop in the number
of implantation sites, viable fetuses, sperm motility and concentration
of cauda epididymal sperms (P<0.001) at the all dose
level (Table 2). The fertility rate of rats reduced upto 90% after the
oral administration of COEtEx (Table 2). Sperm testosterone levels
at all the dose level did not change significantly in the reproductive
and vital organ weight

Table 1: Body and organ weight of control and calendula officinalis extract (50% etoh) treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (gms)</th>
<th>Reproductive and Vital organ weight (mg / 100 gm body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Group I</td>
<td>190.00</td>
<td>±9.01</td>
</tr>
<tr>
<td>Control (vehicle treated)</td>
<td>±8.14</td>
<td>±12.01</td>
</tr>
<tr>
<td>Group II</td>
<td>189.55</td>
<td>±5.06</td>
</tr>
<tr>
<td>150 mg/kg.bwt/day for 60 days</td>
<td>±13.24</td>
<td>±7.12</td>
</tr>
<tr>
<td>Group III</td>
<td>192.60</td>
<td>±4.90</td>
</tr>
<tr>
<td>250 mg/kg.bwt/day for 60 days</td>
<td>±11.51</td>
<td>±6.89</td>
</tr>
<tr>
<td>Group IV</td>
<td>187.51</td>
<td>±5.26</td>
</tr>
<tr>
<td>500 mg/kg.bwt/day for 60 days</td>
<td>±1.14</td>
<td>±4.74</td>
</tr>
</tbody>
</table>

(Mean ± SEM of 10 Animals)

ns = non-significant (P>0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group I

Protein and sialic acid level in testes, epididymis, seminal vesicles
and prostate were significantly lower (P<0.01) in dose dependent
manner after administration of COEtEx (Table 4A). A significant
decrease (P<0.001) was noticed in testicular glycosin and seminal
vesicle’s fructose level (Table 4B). Cholesterol and lipid per
oxidation in testes was significantly higher than control values
(Table 4B). No alteration were observed in liver glycosin and
cholesterol. However, Glutathion in testes showed lower values in
comparison to control group (Table 4B). Hematological parameters
(RBC and WBC counts, Haemoglobin, Haematocrit) (Table 5) and
serological parameters (SGOT, SGPT, Acid phosphates and Alkaline
phosphates) showed non significant change in all the treatment
groups (Table 6).

DISCUSSION
Body weight of rats treated with COEtEx were not altered, but there
was a loss of testicular weight which could be attributed to the loss
of germ cell 26. Decrease weight of accessory sex glands indicate the
atrophy of glandular tissue and diminished secretory ability reflects
the decrease level of testosterone as these organs are androgen
dependent 27. A dose dependent lowering of cauda epididymal
sperrn motility and density suggested an undersupply of testes to
epididymis there by possibly causing impaired epididymal function.
The impaired epididymal function may also be due to the reduced activity of testes, which affect the normal passage
of testicular fluid into the epididymis 28. The principal cells of the
epididymis synthesized proteins that play an important role in
maturation of spermatozoa. 29 In the present study protein were also
reduced due to COEtEx supplementation. Reduced glycogen
reflections a decrease in the number of post-meiotic germ cells which are
thought to be the sites of glucose metabolism. A decrease in
glycogen content in testes indicative of decreased number of post-
meiotic germ cells (spermatids), a site for glucose metabolism 30. An
duced level of Cholesterol may be due to decreased androgen
production, which resulted in accumulation of Cholesterol in testes
and thus impaired spermatogenesis 31. Decreased in Sialic acid
content of testes and sex accessory organs may be correlated with
loss of androgen 32. Fructose is main source of energy required by
spermatozoa. The results from this study indicate COEtEx decreased
the fructose level, because the inhibition of fructose and the
decrease in sperm motility were always correlated 33.

Antioxidants exert their mode of action by suppressing the
formation of reactive oxygen species either by inhibition of enzyme
or by chelating trace element. 34 Administration of COEtEx extracts
caused a significant elevation in TBARS in testes indicating
increased oxidative stress. High level of TBARS might elicit germ cell
apitosis and necrotic change in germ cell 35. Decline in testosterone
may lead to increase in Lipid peroxidation and decline of GSH levels.
A similar increase in Lipid peroxidation with concornitant decline in
GSH leading to impairment of epididymal function have been
reported by Chitra 36.
Table 2: Sperm dynamics and fertility tests of control and *calendula officinalis* extract (50% etoh) treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of males</th>
<th>No of females</th>
<th>No of pregnant females</th>
<th>No of implantation sites/rat</th>
<th>No of viable fetuses</th>
<th>Sperm density (million/ml)</th>
<th>Sperm motility Cauda epididymis</th>
<th>Fertility %</th>
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<tr>
<td><strong>Group I</strong></td>
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<td></td>
</tr>
<tr>
<td>Control (vehicle treated)</td>
<td>10</td>
<td>20</td>
<td>20/20</td>
<td>11.25 ±1.92</td>
<td>8.25 ±1.16</td>
<td>4.55 ±0.43</td>
<td>56.5 ±1.0</td>
<td>79.01 ±1.7</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>150mg/kg.b.wt/day for 60 days</td>
<td>10</td>
<td>20</td>
<td>10/20</td>
<td>7.21* ±2.31</td>
<td>7.01* ±0.78</td>
<td>3.80** ±0.7</td>
<td>40.21** ±1.1</td>
<td>39.57** ±1.88</td>
</tr>
<tr>
<td><strong>Group III</strong></td>
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<td></td>
</tr>
<tr>
<td>250mg/kg.b.wt/day for 60 days</td>
<td>10</td>
<td>20</td>
<td>3/20</td>
<td>4.43** ±1.48</td>
<td>2.34** ±0.61</td>
<td>1.91** ±0.2</td>
<td>18.66** ±0.27</td>
<td>16.05** ±0.24</td>
</tr>
<tr>
<td><strong>Group IV</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 mg/kg.b.wt/day for 60 days</td>
<td>10</td>
<td>20</td>
<td>2/20</td>
<td>2.27** ±1.19</td>
<td>1.09** ±0.32</td>
<td>1.21** ±0.41</td>
<td>10.92** ±6.23</td>
<td>90(-ve)</td>
</tr>
</tbody>
</table>

Mean ± SEM of 10 Animals

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group II

Table 3: Changes in testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) after the treatment of *c.officinalis*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Testosterone (μg/ml)</th>
<th>Follicle Stimulating Hormon (FSH) mIU/ml</th>
<th>Leutinizing Hormon (LH) mIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (vehicle treated)</td>
<td>4.30 ±0.49</td>
<td>0.66 ±0.08</td>
<td>5.17 ±0.45</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
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<tr>
<td>150mg/kg.b.wt/day for 60 days</td>
<td>3.68* ±0.57</td>
<td>0.45* ±0.04</td>
<td>4.47* ±0.42</td>
</tr>
<tr>
<td><strong>Group III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250mg/kg.b.wt/day for 60 days</td>
<td>2.84** ±0.20</td>
<td>0.31** ±0.01</td>
<td>3.12** ±0.21</td>
</tr>
<tr>
<td><strong>Group IV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 mg/kg.b.wt/day for 60 days</td>
<td>1.67** ±0.15</td>
<td>0.18** ±0.08</td>
<td>2.64** ±0.65</td>
</tr>
</tbody>
</table>

Mean ± SEM of 10 Animals

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group II

Table 4 a: Tissue biochemistry of control and *calendula officinalis* extract (50% etoh) treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein mg/gm</th>
<th>Sialic acid (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testis</td>
<td>Epididymis</td>
</tr>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (vehicle treated)</td>
<td>212.43 ±4.15</td>
<td>218.90 ±6.02</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150mg/kg.b.wt/day for 60 days</td>
<td>183.83** ±9.4</td>
<td>195.52** ±5.72</td>
</tr>
<tr>
<td><strong>Group III</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250mg/kg.b.wt/day for 60 days</td>
<td>167.81** ±5.98</td>
<td>180.63** ±4.56</td>
</tr>
<tr>
<td><strong>Group IV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 mg/kg.b.wt/day for 60 days</td>
<td>132.24** ±5.19</td>
<td>139.29** ±2.94</td>
</tr>
</tbody>
</table>

Mean ± SEM of 10 Animals

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group I
CONCLUSION

From the above results it can be concluded that of 50% ethanolic extract of Calendula officinalis(Plant) alters the fertility of rats. Further it reduces the testosterone, androgen dependent parameters and glutathione but increase LPO in testes.

ACKNOWLEDGMENT

The authors are thankful to the Head, Department of Zoology, University of Rajasthan Jaipur and Coordinator of CAS (Centre for Advance Studies) for providing necessary facilities.
REFERENCES


