A studies on Aphrodisiac effect of Madhuca longifolia (Flowers) extract in male Poultry

Mohd. Saif, Rachna Varma, Rishi Kant, Shirish Bhatiya, JP Yadav and Raghavendra Prasad Mishra

Abstract
Regular use of pesticides in our country decrease the fertility day by day that decrease the aphrodisiac effect in poultry. The present study was conducted to investigate the aphrodisiac effect of ethanolic extract of Madhuca Longifolia (Flowers) in male poultry birds. The bird treated with Madhuca (flowers) extract showed positively aphrodisiac effect by increase in value of the semen parameter (sperm conc., live & volume). Sperm concentration was increase in both group 1 and group 2, but in group 2 sperm concentration increase more significantly in comparison to group 1. Live spermatozoa was also increase in group 1 and group 2, in group 2 live sperm percentage was significantly higher than group 1. Semen volume was also increase and found greater in group 2 in comparison to group 1 to control group. On the basis of these findings we can say that Madhuca has positive aphrodisiac effect.

Keywords: poultry, Madhuca, spermatozoa, aphrodisiac

Introduction
Aphrodisiac is the word derived from Aphrodite, the Greek goddess of sexuality, love & beauty. An aphrodisiac is defined as an agent (food or drug) that arouses sexual desire [1]. But present time regular use of pesticides is increasing day by day particularly in third world countries that decrease the fertility continues [2]. India uses approximately 85,000 tonnes of pesticides annually and an increase of 8% is expected every year. The residue of such environmental pollutant remain in soil, water, air, feed & fodder items for a longer period, to contaminate them [3]. Chicken are especially vulnerable to pesticides toxicity because poultry houses are dusted with pesticides that decrease the all the parameter of semen those related the aphrodisiac potential. The logic that there is general resemblance between man and animal with regard to toxicity profile, suggests that animal population can best be utilized as bioindicator of pollution.

Exposure of poultry to chemical pesticides causes health consequences to poultry contributing in great economic loss, while also posing a potential threat to public health due to presence of pesticides in poultry meat, ample evidences exist to suggest that the use of pesticides on crop, in store houses, in poultry houses, the no judicious application for spraying or in dipping solution to prevent ectoparasites, leaves behind in residue causing serious health effect [4, 5]. Chronic exposure of chicks to small amount of OPP leads to deleterious effect on metabolism, immune system and reproductive system of birds [6]. In fact, dairy cattle rearing on drinking water contaminated with sewage reduced their reproductive performance [7].

The exposure of males to pesticides can adversely affect pregnancy outcome through a direct genetic or epigenetic effect of their residues on the male germ cells either during spermatogenesis in the testis or sperm maturation in epididymis or by the direct exposure of oocyte during fertilization to the pesticide residues in the seminal plasma [8, 9]. There is growing evidence regarding the adverse impact of certain pesticide residues on reproductive system and such pesticide residue are known as “reproductive toxicants” or “endocrine disrupters”. These toxicants modulate and or disrupt reproductive hormone milieu by acting at a variety of sites including hypothalamus, pituitary and reproductive organs [10]. During the course of foetal or early neonatal life, any disruption in the differentiation/ multiplication of sertoli cells in fetal testis by the environmental estrogens in detrimental for the adult to produce sperm is determined by the sertoli cells [11, 12, 13].

Materials and Methods
The flowers of Madhuca longifolia were collected from the campus of N.D. University during the month of May & June. The plant material were identified and authenticated with the help of scientist of college of Horticulture. After proper identification flowers was shed dried
powdered and passed through 40 meshed and stored in closed vessel for further use. Madhuca longifolia flowers was used to prepare ethanolic extract. For this purpose absolute alcohol 95% ethanol was used to prepare for extract. Percent yield (w/w) of Madhuca longifolia flowers ethanolic extract was calculated as 42.0%. Percent yield of Madhuca longifolia flowers 45% (w/w) with 95% ethanol [14].

Experimental design

The experimental design for this study is shown in the table No 1. Twenty four male birds about the age 10-12 month were randomly divided into three groups i.e. A, B, C. Each test group comprised of 8 birds along with control as mentioned in table. Doses were given in drinking water approximately 1/10th and 1/5th of LD50 of alcoholic extract of M. Longifolia (Flowers).

Semen collection

The cocks kept on ambient temperature i.e. 30°C and relative humidity i.e. 65% during the study period. One month prior to commencement of semen collection, all cocks were kept in individual cages (32x34x53 cm). All cocks were fed with commercial poultry pellets consisting of 18% crude protein and water was provided ad libitum. Semen samples were collected once a week (Monday) and water was provided ad libitum. Semen samples were collected from each cock replicated on a warm stage. A drop of diluted semen was placed on a preheated slide and a cover slip was used to cover the slide; the cover slip helped to prevent overflow, allowed a uniform film to form, and prevented quick drying of semen [16]. The remnant of the semen in each replicate was measured to evaluate the percentage of live and dead spermatozoa as determined from 250 µL semen, mixed with 50 µL of eosin nigrosin stain to make a thin smear. The smear was air dried for 10 min. At least 200 spermatozoa were examined [17] (400X) under emulsion solution appropriate diluents at a dilution ratio of 1:200 with an eosin solution [16]. The evaluation of sperm motility from the diluted semen was conducted at 400X magnification on a warm stage. A drop of diluted semen was placed on a preheated slide and a cover slip was used to cover the slide; the cover slip helped to prevent overflow, allowed a uniform film to form, and prevented quick drying of semen [17]. The remnant of the semen in each replicate was measured to evaluate the percentage of live and dead spermatozoa as determined from 10µL semen, mixed with 50µL of eosin nigrosin stain to make a thin smear. The smear was air dried for 10 min. At least 200 spermatozoa were examined (400X) under emulsion oil and those with differential morphology were counted. The colour and consistency of the semen were evaluated visually, including varieties that were creamy, grainy, bloody, watery, or contaminate. The following parameters were recorded for the evaluation of aphrodisiac effect of alcoholic extract of Madhuca longifolia (Flowers) in male poultry birds.

Table 1: Experimental design for evaluation of effect of extract of Madhuca (flower) in male poultry birds.

| Group | Extract/drug | No. of animal | Dose of Extract (mg/kg b.wt.) | Duration
|-------|--------------|---------------|-----------------------------|----------
| A     | Control (G0) | 8             | -                           | 28 days  |
| B     | 1/10th of LD50 of M. longifolia (flowers) (G1) | 8 | Daily single Dose | 28 days |
| C     | 1/5th of LD50 of M. longifolia (flowers) (G2) | 8 | 200 | 28 days |

Semen evaluation

Sodium citrate and egg yolk were prepared and used as an extender in this study. The volume of the ejaculated semen from each cock was measured using a graduated test tube. The pH was determined using a pH meter. Spermatozoa concentration was determined using the haemocytometer method, and the technique involved in mixing semen with appropriate diluents at a dilution ratio of 1:200 with an eosin solution [16]. The evaluation of sperm motility from the diluted semen was conducted at 400X magnification on a warm stage. A drop of diluted semen was placed on a preheated slide and a cover slip was used to cover the slide; the cover slip helped to prevent overflow, allowed a uniform film to form, and prevented quick drying of semen [17]. The remnant of the semen in each replicate was measured to evaluate the percentage of live and dead spermatozoa as determined from 10µL semen, mixed with 50µL of eosin nigrosin stain to make a thin smear. The smear was air dried for 10 min. At least 200 spermatozoa were examined (400X) under emulsion oil and those with differential morphology were counted. The colour and consistency of the semen were evaluated visually, including varieties that were creamy, grainy, bloody, watery, or contaminate. The following parameters were recorded for the evaluation of aphrodisiac effect of alcoholic extract of Madhuca longifolia (Flowers) in male poultry birds.

Statistical study

In this study statistically, analysis of variance was applied. Completely randomized design was used and significant differences was analysed at 5% level of significant. Comparative study among group 1 and group 2 to control group was done by Das [18].

Table 2: Effect of ethanolic extract of Madhuca longifolia (Flowers) at 7 days on below various semen parameter on male poultry birds - (n=8) (mean ± S.E.,)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated with oral dose/10 LD50 of Madhuca</th>
<th>Treated with oral dose/5 LD50 of Madhuca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G0</td>
<td>G1</td>
<td>G2</td>
</tr>
<tr>
<td>Concentration of spermatozoa (10^9/Sperm/ml)</td>
<td>2.62±0.03^c</td>
<td>2.88±0.03^d</td>
<td>3.01±0.03^d</td>
</tr>
<tr>
<td>Live spermatozoa %</td>
<td>97.61±0.35</td>
<td>98.09±0.30</td>
<td>98.38±0.30</td>
</tr>
<tr>
<td>Volume (ejaculate/ml)</td>
<td>0.03100±0.05</td>
<td>0.0333±0.05</td>
<td>0.0345±0.05</td>
</tr>
</tbody>
</table>

Values with different superscripts in a differ significantly (P<0.05).

Table 3: Effect of ethanolic extract of Madhuca longifolia (flowers) at 14 days on below various semen parameter on male poultry birds - (n=8) (mean ± S.E.,)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated with oral dose/10 LD50 of Madhuca</th>
<th>Treated with oral dose/5 LD50 of Madhuca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G0</td>
<td>G1</td>
<td>G2</td>
</tr>
<tr>
<td>Concentration of spermatozoa (10^9/Sperm/ml)</td>
<td>2.62±0.03^c</td>
<td>2.93±0.03^d</td>
<td>3.09±0.04^e</td>
</tr>
<tr>
<td>Live Spermatozoa (%)</td>
<td>97.61±0.35</td>
<td>98.35±0.34</td>
<td>98.74±0.35</td>
</tr>
<tr>
<td>Volume (ejaculate/ml)</td>
<td>0.031±0.05</td>
<td>0.034±0.05</td>
<td>0.035±0.05</td>
</tr>
</tbody>
</table>

Values with different superscripts in a differ significantly (P<0.05).
Table 4: Effect of ethanolic extract of *Madhuca longifolia* (Flowers) at 28 days on below various semen parameter on male poultry birds - (n=8) (mean ± S.E.).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control G0</th>
<th>Treated with oral dose/10 LD₅₀ of Madhuca G1</th>
<th>Treated with oral dose/5 LD₅₀ of Madhuca G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of spermatozoa (10⁹ sperm/ml)</td>
<td>2.62±.029&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.01±.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.14±.04&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Live spermatozoa (%)</td>
<td>97.61±.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>98.58±.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>99.01±.34&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Volume(ejaculate/ml)</td>
<td>0.31±.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.34±.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.37±.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different superscripts in a differ significantly (P<0.05).
Effect of these extracts on various semen parameters (sperm conc., live and volume) at 7 days are presented in table. No. 2 and showed by graph no. 1, 2, and 3. The bird treated with Madhuca flowers extract showed positively aphrodisiac effect by increase in value of all the semen parameter. Sperm concentration was increase in both group 1 and group 2 but in group 2 sperm concentration increase more significantly in group 1. Live spermatozoa was also increase in group 1 and group 2, in group 2 live sperm percentage was significantly higher than group. Semen volume was also increase and found greater in group 2 in comparison to control group.

After 14 days effect of Madhuca represented in table no 3 and showed by graph no. 4, 5 and 6. At 7 days both dose (1/5 and 1/10 LD50) of Madhuca longifolia flowers extract had positively significant result in all the parameters. In group 2 where 1/5 dose of Madhuca was given result was highly positively significant in comparison to group 1.

After 28 days effect of Madhuca represented in table no. 4 and showed by graph no. 7, 8, and 9. At the 28 days both doses of Madhuca were continue to increase the value of all the parameter (sperm conc., live and volume). The animal of group 2 exhibited more positively significant in comparison of group 1.

Comparing 1/10th & 1/5th of LD50 of Madhuca, 1/5th showed better result i.e. better aphrodisiac effect. These finding suggests that Madhuca has positive aphrodisiac effect. Similar findings were observed at 14 and 28 days, but there was a continuous improvement recorded in all the parameters.

Conclusions
On the basis of these findings we can say that Madhuca has positive aphrodisiac effect. There is a scope of further investigation regarding Madhuca flower extract.

Acknowledgments
This research work was financially supported by the College of Veterinary science, University of kumarganj Faizabad, India.

Competing Interests
The authors declare that they have no competing interests.

References


19. OECD guidelines for the testing of chemical acute oral toxicity test up and down procedure (UDP) 425 adopted, 2008.