Evaluation of aphrodisiac potential on the basis of semen motility of poultry by feeding the extract of Madhuca longifolia (Flowers)

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Abstract
The present study was conducted to investigate the aphrodisiac potential of ethanolic extract of Madhuca Longifolia (flowers) in male poultry birds. The bird treated with Madhuca (flowers) extract showed positively increase in value of the semen parameter (General and progressive motility). General and progressive motility was also increase more significantly in group 2 in comparison to group 1 and control group. On the basis of these findings we can say that Madhuca has positive aphrodisiac potential. Madhuca 1/10 dose of LD50 less effective in comparison to 1/5 dose of LD50 and 1/5 dose of LD50 showed high motility in comparison to control group of male poultry birds. Aphrodisiac potential increases and less effective 1/10 dose of LD50 of Madhuca on 7th, 14th and 28th day in comparison to 1/5 dose of LD50. Madhuca flower give energy help in increasing the semen motility and then become high quality and murine is absent in flower that is toxic.

Keywords: Madhuca, poultry, motility, spermatozoa

Introduction
Present time regular use of pesticides is increasing day by day particularly in third world countries that decrease the fertility continues [1]. 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 [2]. 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.

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 [3, 4]. Chronic exposure of chicks to small amount of OPP leads to deleterious effect on metabolism, immune system and reproductive system of birds [5]. In fact, dairy cattle rearing on drinking water contaminated with sewage reduced their reproductive performance [6].

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 [7, 8]. 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 [9]. During the course of foetal or early neonatal life, any disruption in the differentiation/multiplication of sertoli cells in fetal testis by the environmental estrogen in detrimental for the adult to produce sperm is determined by the sertoli cells [10, 11, 12].

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.[13]

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 (32×34×53 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). Semen collection was done from testes to distal region of duct’s deferens varies from 1-4days.[14] First, the cloacae area was cleaned. The back and tail feathers and the abdominal region were stroked gently and repeatedly, which resulted in erection of phallus. Semen was ejaculated after slight pressure was applied to the inverted cloacae. The semen was carefully collected in a test tube and placed in a water bath maintained at 37 °C prior to evaluation.

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. 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 pre-heated 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.[15]. 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 adopted. 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[17].

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

| Group | Extract/drug | No. of animal | Dose of Extract (mg /kg b. wt.) | Duration |
|-------|--------------|---------------|---------------------------------|----------|
| A     | Control (G0) | 8             | 700 Daily single Dose           | 28 days  |
| B     | 1/10th of LD50 of M. longifolia (flowers) (G1) | 8 | 100 Daily single Dose | 28 days |
| C     | 1/5th of LD50 of M. longifolia (flowers) (G2) | 8 | 200 Daily single Dose | 28 days |

Result & Discussion
In this study Madhuca longifolia flowers extract was given @ 100 mg/kg b. wt (1/10th of LD50) and 200 mg/kg b. wt (1/5th of LD50) daily single dose (OECD, 2008).

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.,).

| Parameter | Control (G0) | Treated with oral dose 1/10 LD50 of Madhuca | Treated with oral dose 1/5 LD50 of Madhuca |
|-----------|--------------|--------------------------------------------|------------------------------------------|
| G0        |              |                                            |                                          |
| General motility (%) | 59.56±5.55 | 65.53±6.16 | 68.59±6.45 |
| Progressive motility (%) | 31.13±9.76 | 32.68±1.02 | 34.24±1.07 |

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.,).

| Parameter | Control (G0) | Treated with oral dose 1/10 LD50 of Madhuca | Treated with oral dose 1/5 LD50 of Madhuca |
|-----------|--------------|--------------------------------------------|------------------------------------------|
| G0        |              |                                            |                                          |
| General motility (%) | 59.56±5.55 | 66.76±1.91 | 70.28±1.65 |
| Progressive motility (%) | 31.13±7.97 | 33.02±1.05 | 34.86±1.09 |

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.,).

| Parameter | Control (G0) | Treated with oral dose 1/10 LD50 of Madhuca | Treated with oral dose 1/5 LD50 of Madhuca |
|-----------|--------------|--------------------------------------------|------------------------------------------|
| G0        |              |                                            |                                          |
| General motility (%) | 59.56±5.55 | 68.46±1.02 | 71.48±1.16 |
| Progressive motility (%) | 31.13±9.76 | 34.24±1.07 | 35.81±1.11 |

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

Effect of these extracts on various semen parameters (General and progressive motility) at 7 days are presented in table. No. 2.

The bird treated with Madhuca flowers extract showed positively aphrodisiac effect by increase in value of various semen parameters. General and progressive motility was
increase in both group 1 and group 2 but in groups 2 General and progressive motility increase more significantly in group 1. At 7 days both dose (1/5 and 1/10 LD50) of Madhuca longifolia flowers extract had positively significant result in all the parameters. After 14 days effect of Madhuca represented in table no 3. 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. At the 28 days both doses of Madhuca were continue to increase the value of various semen parameter (General and progressive motility). 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 [20]. Similar findings were observed at 14 and 28 days, but there was a continuous improvement recorded in various semen parameters.

**Conclusions**

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

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**Competing Interests**

The authors declare that they have no competing interests.

**References**

1. Forget G. 1991. Pesticide and third world. J Toxicol Environ health 1991;32:11-31.
2. W H O. Public health impact of pesticides used in Agriculture, WHO, Geneva 1990.
3. Pal AK, Kushwah HS. An assay of brain acetyl cholinesterase activity vis-à-vis Malathion toxicity in chicken. Indian Vet Med J. 1998;22:233.
4. Pal AK, Kushwah HS. Quantative biological lesions of malathion dipping in domestic fowl Gallus domesticus. Asian-Austra J Anim Sci, 2000;13:285–90.
5. Garg, UK, Pal AK, Jha GJ, Jadhao SB. Haematotoxity with synthetic pyrethroid. Immunopharmacol 2004a;(13):1700-1722.
6. Meijer G, Debree AL, Wagenaar JA, Spoelstra SF. Sewerage overflows put production and Fertility of dairy cows at risk. J. Environ. Qual 1999;28:1381-83.
7. Bonde JP, Joffe, Apostoli M, Dale A, Kiss P, Spano M et al. Sperm count and chromatin structure in men exposed to inorganic lead. Lowest adverse effect level. Occ. Environ. Med 2002;59:234-42.
8. Colborn T, Frederick S, Vom S, Ana MS. Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife and Humans. Environmental Health Perspectives. 1993;101:378-384.
9. Sweeny T, Nicol L, Roche JF, Brooks AN. Maternal exposure to octylphenol suppresses ovine fetal follicle stimulation hormone secretion, testis size and Sertoli cell numnder. Endocrinology 2000;141:2667-73.
10. Sharpe RM, Skakkebaekne. Are oestrogen involved in falling sperm counts and disorders of the male reproductive tract? The lancet 1993;341:1392-95.
11. Pocar P, Brevini TA, Perazzoli F, Modina S, Gandolfi F. Cellular and molecular mechanisms mediating the effects of polychlorinated biphenyls on oocyte developmental competence in cattle. Mol Reprod. Dev 2001;60:535-41.
12. Boerjan ML, Freijngael S, Rhind SM, Meijer GAL. The potential reproductive effect of exposure of domestic ruminants to endocrine disrupting compound. Ani. Science 2002;74:3-12.
13. Katiyar Swati, Katiyar Manish, Tandon Amol, Chandekar, Neeraj, Upmanyu. Pharmacognostic standardization phytochemical investigation and anthelmetics evaluation of extract of Madhuca indica. J.F. (GME) Flower. Pharmacology online 2011;3:892-903.
14. Munro S. Functional change in fowl sperm during their passage through the excrcnt ducts of the male. J Exp. zool, 1938, 71-92.
15. Brillard JP, Mc Daniel. The reliability and efficacy of various methods for estimating, spermatozoa conc. Poult. sci 1985;64:155-158.
16. Birkhead TR, Martinez JG, Burke T, Forman DP. Sperm mobility determines the outcome of sperm competition in the domestic fowl. Proc. Biol. Sci 1999;266:1759-1764.
17. Das Statistical Analysis 2000.
18. OECD guidelines for the testing of chemical acute oral toxicity test up and down procedure (UDP) 425 adopted 3 October 2008.
19. Thakur M, Chauhan SN, Bhargava S, Dixit VK. “A comparative study on aphrodisiac activity of some ayurvedic herbs in male albino rats,” Archives of Sexual Behaviour, 2009;38:1009-1015.