Contraceptive Evaluation of Oil Extract of Seeds of *Mimosa Pudica* (L) in Male Wistar Rats

Raghunath D.Pokharkar, Rajeshwari .K.Saraswat, Minal G. Kanawade

Department of Chemistry, S.N. Arts, D.J.M.Commerce & B.N.S.Science College, Sangamner 422605

Summary

The present study evaluates the contraceptive effect of the oil extract of seeds of Mimosa pudica (L) (Leguminosae) in male albino rats. The oil extract of Mimosa pudica was administered 0.250 ml/kg body weight for 45 days. Sperm motility and density in cauda epididymis were assessed. Biochemical indices, toxicological profile and fertility rate have been recorded. The sperm motility and density were significantly reduced and protein in the epididymis was significantly decreased. The RBC, WBC, Platlets, MCH, MCV, MCHC, MPV, PCT, RDW etc. were studied. The oil extract of mimosa pudica causes reversible spermicidal effect.

Key words: Mimosa-pudica, male rats, spermicidal activity, testosterone.

Correspondence to:

R.K.Saraswat- Department of Chemistry, S.N. Arts, D.J.M.Commerce & B.N.S.Science College, Sangamner 422605, Maharashtra.India. E-mail:- <u>shaileshza@hotmail.com</u>, <u>pef@rediffmail.com</u>

Introduction

The world population is expected to rise from the current 6.5 billion to 9.1 billion by 2050, the UN says. Population is people and people matter (Cohen). Juice of Mimosapudica leaves used in sinus, sores, piles and fistula: paste applied to glandular swellings and hydrocele. The root is bitter and acrid: cooling, Vulnerary, alexipharmic; cures kapha, biliousness, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, fatigue, asthma, leucoderma, and diseases of the blood¹. Herbal drugs have been used as medicines for treatment of for a range of diseases since ancient time.

Pokharkar et al.

Inspite of great advances observed in modern medicine in recent decades, plants still make an important contribution to health care². The objective of the present work was to investigate the spermicidal effect of the oil extract of the seeds of mimosa-pudica is an attempt to establish the traditional use. In many places, Mimosa-pudica has becomes a trouble some weed. Application of MCPA at 4 to 6 lb/acre affords effective control ³.

Methods

Plant collection and extraction

The seeds are collected from Western Ghats of Maharastra. It was identified and authenticated by experts. The seeds were dried and powdered then sesame oil extract is prepared.

Animals

Healthy, colony bred adult mal albino rats of wistar strain, weighing between 240 to 260 grams were used for various experiments. The rats were housed in polyurethane cages under room temperatures. Rat feed (amrut) and tape water provided ad libitum. Body weight of each animal in all groups was measured form three days to see the possible body weight changes.

Experimental design

Male rats are of proven fertility were divided into three groups of six rates each. The daily dose of oil extract was administered to each on -

Group 1 – Control rats received only vehicle

Group 2 – Rats treated with oil extract at 0.250 ml/kg body weight per day for 45 days.

Group 3 - Rats treated with oil extract at 0.250 ml/kg body weight per day for 45

days. The recovery is tested after 60 days.

Autopsy schedule -24 hours of the last dosing, the males were weighed and autopsied under ether anesthesia and the reproductive organs i.e. testes and cauda epididymis weighed.

Sperm density and motility – The sperm density was assessed in cauda epididymis. A known amount of cauda epididymal tissue was taken in physiological saline and teased gently to release the spermatozoa. The suspension was used for the counting the number of sperms.

Pokharkar et al.

Parameter studied – General behavior, body weight, food and water intake were noted daily during the treatment period all the animals of group 1 &2 were sacrificed after the treatment for further studies. Blood was collected by cardiac puncture under light anesthesia for the estimation of different blood parameters

Results

Body and organ weight – The oral administration of mimosa-pudica seed oil extract didn't cause significant change in the body weight, decrease the weight of cauda epididymis and testes.

Table 1-Comparison of mean serum testosterone level, mean body weight and mean absolute weight of testes and cauda-epididymis in control, experimental and recovery group (n=6)

Parameter	Group 1	Group 2	Group 3
Body weight (gm)	250 ± 2.00	273.33 ± 3.08	270.33 ± 2.33
Testes(mg)	2881.66 ± 19.22	2478 ± 2.92	2867.66 ± 3.07
Cauda epididymis(mg)	480 ± 5.75	438.16 ± 3.41	474 ± 3.26
Sperm count(million/ml)	88.33 ± 6.14	12.00 ± 0.57	82.5 ± 2.29
Serum testosterone (ng/ml)	2.43 ± 0.01	0.29 ± 0.01	2.25 ± 0.07



- Fig.: 1-Comparison of testes weight of group 1, 2, 3-
 - A: Weight of testes of group 1.
 - B: Weight of testes of group 2
 - C : Weight of testes of group3



Fig.: 2- Comparison of cauda-epididymis weight in group 1, 2, 3-

- A: Weight. of cauda-epididymis of group 1.
- B: Weight. of cauda-epididymis of group 2
- C : Weight. of cauda-epididymis of group 3.



Fig.: 3-Comparison of sperm count in group1, 2, 3-

- A: Sperm count in group1
- B: Sperm count in group2
- C: Sperm count in group3



- Fig.: 4-Comparison of testosterone level in group1, 2, 3-
 - A: Testosterone level in group1
 - B: Testosterone level in group2
 - C: Testosterone level in group3

Blood cell counts-All the values of blood parameters Red blood cells (RBC), White blood cells (WBC), Platel. (Platelets count), Lym. (Lymphocytes count), Mon. (Monocytes count), Gran. (Granulocytes count) and % of Lymphocyte, Monocytes , Granulocytes are counted..

Table2- Effect of Oil extract of seeds of Mimosa-pudica on blood cell counts (RBC,WBC, Platel., Lym, Mon., Gran.)

Parameters	Group 1	Group 2	Group 3
RBCs x 10 ⁶ /mm ³	8.22 ± 0.24	7.23 ± 0.19	8.16 ± 0.06
WBCs x 10^3 /mm ³	6.81 ± 0.20	5.33 ± 0.23	6.71 ± 0.21
Platlets x 10 ³ /mm ³	645 ± 2.76	533.46 ± 4.11	6.40 ± 4.70
Lym. x 10^3 /mm ³	5.58 ± 0.17	4.9 ± 0.21	5.46 ± 0.04
Mon. x 10^3 /mm ³	1.06 ± 0.00	0.4 ± 0.04	0.98 ± 0.03
Gran.x 10 ³ /mm ³	2.93 ± 0.08	1.96 ± 0.06	3.02 ± 0.04
% Lymphocytes	58.22 ± 1.09	67.31 ± 1.18	57.71 ± 0.49
% Monocytes	11.11 ± 0.19	5.52 ± 0.60	10.35 ± 0.33
% Granulocytes	30.65 ± 0.99	27.14 ± 1.03	31.71 ± 0.39

Some more blood parameters-Values of blood parameters like Hemoglobin(HGB),Hematocrit(HCT),Mean corpuscular volume (MCV),Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration(MCHC),Red cell distribution width(RDW),Mean platelet volume (MPV),Platelet distribution width (PDW),Platecrit (PCT)-

Parameters	Group 1	Group 2	Group 3	
HGB (g/dl)	13.26 ± 0.19	11.90 ± 0.12	13.20 ± 0.10	
HCT %	48 ± 0.48	45.83 ± 0.31	46.66 ± 2.90	
MCV (fl)	58.2 ± 0.37	53.2 ± 0.19	58.13 ± 0.34	
MCH (pg)	16.3 ± 0.26	16.35 ± 0.15	15.95 ± 0.36	
MCHC(g/dl)	28.1 ± 0.28	30.18 ± 0.47	28.2 ± 0.31	
RDW (%)	15.58 ± 0.16	16.51 ± 0.19	15.45 ± 0.21	
MPV (fl)	10.7 ± 0.22	8.91 ± 0.15	10.81 ± 0.27	
PDW (%)	15.65 ± 0.17	16.95 ± 0.14	15.62 ± 0.23	
PCT (%)	0.316 ± 0.007	0.467 ± 0.002	0.350 ± 0.017	

Table 3- Effect of Oil extract of seeds of Mimosa-pudica on some blood parameters-(HGB, HCT, MCV, MCH, MCHC, RDW, MPV, PCT).

Liver function enzymes-Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) Alkalyne phosphatase (Alk.PO₄), Serum total protein (TP) were also studied

Parameters	Group 1	Group 2	Group 3
SGOT (u/l)	171 ± 2.76	186.83 ± 1.42	177.16 ± 3.06
SGPT(u/l)	154.33 ± 1.20	78.16 ± 1.75	152.33 ± 2.80
Alk. $PO_4(u/l)$	159.33 ± 1.97	73.16 ± 2.28	160 ± 1.65
TP (g/dl)	8.56 ± 0.15	6.31 ± 0.15	8.15 ± 0.10

Table4-- Effect of Oil extract of seeds of Mimosa-pudica on liver function enzymes-

Discussion

The study was deliberately conducted on an animal model. Throughout the experiments, all rats showed nearly equal weight gain. The weight of group 2 rats increases slightly. In the absence of any known pathology, testis weight is highly related to daily sperm production.⁴. The weight of the testis is one of the markers of a possible alteration in androgen status. A decrease in testicular weight in experimental rats is most likely due to decreased level of serum testosterone, as androgen exerts its major role in sex-organs⁵.

One of the causes of decrease absolute testicular weight and GSI (a better way to assess the damage to the testes in relation to the body) may be an effect of poor nutrition or decrease food intake^{6, 7}. Since both the groups were supplied with the same standard rat diet and their food was also satisfactory, there is less likelihood to poor nutrition being the cause of decrease testicular weight in experimental group.

Literature has shown that oral ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters^{8, 9}. These alterations could either be positive or negative. In this study, most of the effects of blood parameters recorded for the extract were slightly negative except for some blood parameters.

Last but not the least, literature review strongly points towards the importance of pituitaries in maintaining testicular size and weight. A sudden increase of rat serum gonadotropins at the time of birth is followed by a rapid increase of absolute and relative testicular weights¹⁰.

Findings of this study revealed that hematological variables such as WBC, RBC, HCT and PLT decreased slightly in treated group when compared with control group. Decreasing leukocytes counts were observed in our study is comparable with findings of Heinze et al. study¹¹. Androgen, or more specifically testosterone, is widely utilized to treat erectile dysfunction¹².

In table 1, there is slight change in the difference of body weight, but the weight of testes and cauda-epidiodymis decreases significantly. This directly indicates that due to this the testosterone level also decreases. Sperm count is also decreased in treated group as compared to control group. These changes show the spermicidal activity. There is also graphical representation of standard error mean value of different parameters- weight of testes, weight of cauda-epididymis, sperm-count and testosterone level.

In table 2, we have blood cell count- RBC, WBC, Platelets, Lymphocytes, Monocytes and Granulocytes count. These all parameters decrease slightly. So, these don't show any toxic effect to animal.

In table 3, blood parameters HGB (Hemoglobin), HCT %, MCV (Mean corpuscular volume), MPV (Mean platelet volume) are decreases slightly. Simultaneously MCHC (Mean corpuscular hemoglobin concentration), RDW (Red cell distribution width), PDW (Platelet distribution width), MCH (Mean corpuscular hemoglobin), Platecrit (PCT) are increasing slightly.

In table 4 - SGOT (Serum glutamic oxaloacetic transaminase) increases, but SGPT (Serum glutamic pyruvic transaminase) and alkaline phosphates (Alk.PO₄)decreases. Serum total protein is also decreasing. But this doesn't show any type of abnormalities in animal. These above SGOT, SGPT are liver enzymes, which affect the liver.

The protein synthesis and concentration in the accessory sex organs are androgen dependent. Administration of antiandrogens caused a significant decrease in the protein concentration after testosterone therapy¹³.

The sperm count (concentration) of group 1 (88.50 x 10^6) was significantly higher than group 2(12.00 x 10^6) value.

It can be therefore concluded that dose of 0.250 ml/ kg body weight of oil extract of Mimosa- pudica seeds for as long as 45 days will have a deleterious effects on the reproductive function of the male wistar rat.

Statistical analysis- For all the quantitative data, generated by the experiments, differences between individual groups were analyzed for statistical significance using appropriate test. The results were expressed as Mean \pm SE.

Conclusions

From above results we can conclude that there are some changes in treated group.

Slight increase in-Body weight, Mean corpuscular hemoglobin concentration(MCHC),Red cell distribution width(RDW) Platelet distribution width (PDW),Platecrit (PCT), Mean corpuscular hemoglobin (MCH), Serum glutamic oxaloacetic transaminase (SGOT).

Slight decrease in- Red blood cells (RBC), White blood cells (WBC), Platel.(Platelets count), Lym.(Lymphocytes count), Mon. (Monocytes count), Gran.(Granulocytes count), Hemoglobin(HGB), Hematocrit(HCT), Mean corpuscular volume (MCV), Mean platelet volume (MPV), Serum total protein (TP).

Major change in-Weight of cauda-epididymis, weight of testes, testosterone level, sperm count, serum glutamic pyruvic transaminase (SGPT) and alkaline phosphates (Alk.PO₄). It means that the oil extract of Mimosa-pudica seeds exert a significant role to decrease the testosterone level, just like the oil extract of Butea monosperma¹⁴. it shows spermicidal activity.

References

1. Ayurveda Kala Mandiram. www.ayurvedakalamandiram.com

2. Shu, Y.Z.; J Nat Prod., 61: 1053-1071 (1998).

Brenan, Kew Bull., 161; Use of Leguminous Plants, 226; Ram Gopal, Indian Fmg, N.
S., 1954-55, 4(10), 23. 1995.

4.Zindy F. Besten WD, Chen B, Rehg JE, Latres E, Barbacid M, et al. Control of Spermatogenesis in Mice by the cyclin D- Dependent Kinase inhibitors $_{p}18^{Ink4c}$ and $p^{19Inkud}$, Mol Cell Biol;21:3244-55, **2001**

5. Simanainen U, McNamara K, Davey RA, Zajac JD, Handelsman DJ. Severe subfertility in mice with androgen receptor inactivation in sex accessory organ but not in testis, Endocrinology; 149:3330-8, **2008**

6. Rehm S, White TE, Zahalka EA, Stanisalus DJ, Boyce RW, Wier, P. J. Effects of food restriction in testis and accessory sex glands in maturing rats. Toxicol Pathol; 36: 687-94, 2008

7. Faucher CJ, Berger M, Turckheim MD, Jean GVC. Effect of preweaning under nutrition on testicular development in male mice. International Journal of Andrology; 5: 627-35, **2008**

8..Ajagbonna OP, Onifade KI, Suleiman U. Haematological and Biochemical changes in rats given extract of Calotropis procera. Sokoto Journal of Vaterinary Sciences; 1:36-42, 1999

9. Ofuya ZM, Ebong OO. Plasma ascorbic acid levels in adult females in Port-Harcourt, South Eastern Nigeria. West African Journal of Pharmacology and Drug Research; 12:32-36, **1996**

10. Corbier P, Kerdelhue B, Picon R, Roffi J. Changes in testicular weight and serum gonadotropin and testosterone levels before, during, and after birth in perinatal rat. Endocrinology; 103: 1985-91, **1978**

11. Heinze, W., H. Nattermann and Z. Wiss, Humboldt –univ Berlin. Math- Naturwiss Reihe, 33(5): 513-517, **1984**

12. www.testosterones. Com (access in August 2005).

13. Brooks DE, Higgins SJ. Characterization and androgen dependence of proteins associated with luminal fluid and spermatozoa in rat epididymis. J Reprod Fertil; 59: 363-75, **1980**.

14. Pokharkar R. D., Saraswat R. K and Pokharkar M. R., Contraceptive efficacy of Butea monosperma seed extract in Wistar rat, Pharmacologyonline 2:722-726(**2009**).