Contraceptive Evaluations of Oil Extract of Seeds of *Abrus Precatorius* (L) in Male Albino Rats

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Summary

The present study was designed to evaluate the contraceptive effect of oil extract of seeds of Abrus precatorius (L.) (Fabaceae) in wistar rats. The body and organ weights, cauda epididymal biochemical indices etc. have been recorded. Treatment caused a significant decrease in caudal sperm count and testosterone level. A reduction in the testosterone level was suggestive of reduced reproductive performance. Reversibility test showed that the antifertility effect of Abrus precatorius was completely reversible on withdrawal of the drug. The result suggests that the extract induces reversible antifertility effect. Condom; vasectomy and withdrawal are the only male contraceptive devices available with less assurance for men. It is necessary to use biologically active botanical substances or fertility regulating agents of plant origin.

Key words: Abrus seeds, contraception, hematology, serum.

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Introduction

The only male specific contraceptive methods currently available are withdrawal, condoms and vasectomy. As concerns regarding side effects and convenience of these existing methods prevent their universal acceptance.[1,2] The epididymis plays an important role in sperm development and sperm maturation is dependent on the unique luminal environment of the epididymis including specific proteins synthesized and secreted by the epididymal epithelium[3,4] .The research in to the efficacy of herbs used in traditional veterinary practice would be useful in establishing standard dosages for herbal preparations and to investigate their toxicity.[5] The studies on the male antifertility effects of various medicinal plants have aroused much interest[6-9] .The world population was 2.55 billion in 1950 and was 6.45 billion in April 2005 is still increasing at rate of 1.15% annually, World Health Organization (WHO) study in man found that between 45 and 71 percent of man would welcome a safe ,reversible ,convenient, non-surgical contraceptive ,which could be used separately from intercourse[10].

Ratnasooriya et al.have reported sperm antimotility properties of a seed extract of Abrus precatorius.They examined the inhibitory effect of a methanol extract of Abrus precatorius seeds on the motility of washed human spermatozoa[11]. During the past few decades sporadic attempts have been made by various investigators to develop male contraceptive agents from various antifertility plants available in their locality. Various medicinal plant extracts have been tested for their antifertility activity both in male and female[12] .Some of these plants had spermicidal effects, others caused reduction in the sperm counts and altered the mobility of the sperms. Some of them caused testicular change and altered hormone levels[13, 14] .Many chemicals and pesticides also affect the male fertility [15].These result prompted us to screen various plants in our locality. So by the survey of the plants in Western Ghats of Maharashtra[16] we have selected one of the plant Abrus precatorius.

Methods

The seeds of Abrus precatorius are purgative, emetic, tonic, antiphylogistic, aphrodisiac and used in nervous disorders; abortifacient; paste as local application in stiffness of shoulder joints, sciatica and paralysis. Taken internally by women, the seed disturbs the uterine functions and prevents conception. The powdered seeds are taken as snuff in cases of violent headache arising from cold[17] .The seeds are collected from Western Ghats of Maharastra. It was identified and authenticated by experts. The seeds were dried and powdered. Than the oil extract is prepared.

Over three century laboratory animals have been used in different research activities[18-21].Healthy, colony bread male albino rats of wistar strain weighing 240-260 Gms were housed in polyurethane cages with wire mesh tops and rice husk bedding and maintained on standard pellet diet (Amrut feed) with water ad libitum. Animals were acclimatized at a room temperature of 26-28° C. General behaviour, food and water intake were noted daily during the treatment period.

Hematological examination- When the experiments had expired, each of the rats of the experiments and the control groups were anaesthetized with ether. Blood were taken from heart and collected in tube containing EDTA for hematological investigations. The blood parameters such as 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 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) etc. examined.

Biochemical estimation-The blood samples without anticoagulant were left at room temperature for 45 minutes for clotting, then centrifugiated at about 3000 rpm for 15 minutes and the serum was separated for biochemical analyses. Serum alkaline phosphates (Alk. PO_4) activity and the level of total protein (TP) were determined. Serum testosterone level was estimated by radioimmunoassay. The sperm count was determined from the cauda-epididymis using a Heamatocytometer Neubauers chamber.

Results

The results are summarized in Table 1 to 4 as the mean values and standard deviations (X ± S.D.) with minimum and maximum.

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: The body weights, weight of testes, cauda epididymis, sperm count and testosterone level-

Groups	Control (n = 6)	Treated (n = 6)	Recovery (n=6)
Variables	(X ± S.D.) Minima-Maxima	(X ± S.D.) Minima-Maxima	(X ± S.D.) Minima-Maxima
BW (g)	250.00 ± 4.89 (242-256)	251.83 ±5.38 (244-258)	256.33 ± 8.11 (240-264)
Testes (mg)	2881.66 ± 4.36 (2877-2889)	2463.00 ± 11.62 (2448-2478)	2877.16± 8.11 (2867-2890)
Cauda-epididymis (mg)	480.00± 14.08 (460-496)	411.33 ± 3.72 (406-412)	475.00 ± 8.46 (464-486)
Testosterone (ng/ml)	2.43 ± 0.03 (2.38-2.48)	0.41± 0.04 (0.33-0.46)	2.42 ± 0.13 (2.24-2.58)
Sperm count (million/ ml)	88.33± 15.04 (68-104)	16.33 ± 3.44 (12-22)	85.33± 11.36 (68-98)

The mean body weights of treated and controlled 251 and 250 gm. respectively did not differ significantly. But in recovery group it increases with time. As shown in Table 1, only the differences obtained from Mean values of weight of testes, cauda epididymis, testosterone level and sperm count were significant. The weight of testes also decreased from 2881.66 to 2463 mg. The weight of cauda-epididymis also decreased from 480 mg to411.33 mg.Testosterone level decreases from 2.43 to 0.41(ng/ml).Sperm count is decreased from 88.33 to 16.33 (million/ ml). But these all again recovers in recovery group.

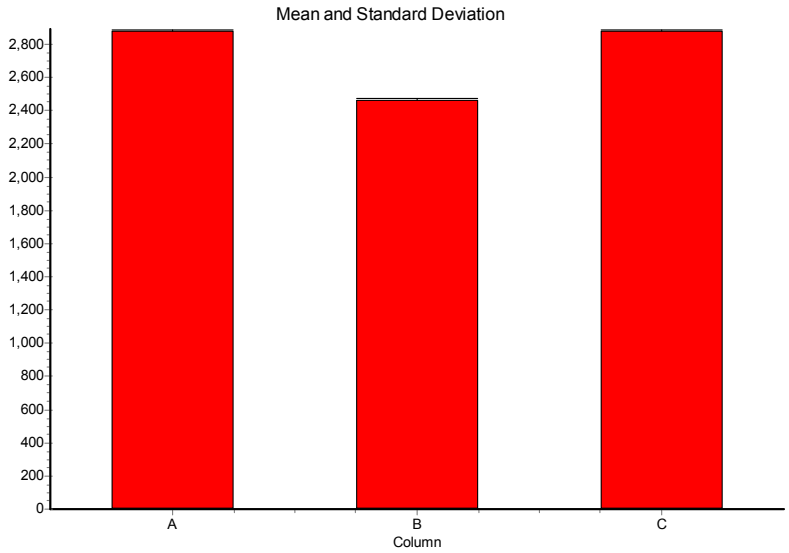

Figure 1: Weight of testes in three groups of rats. Levels of significance values are (mean ± S.D.; n = 6) A: Weight of testes of control group , B: Weight of testes of treated group , C: Weight of testes of recovery group

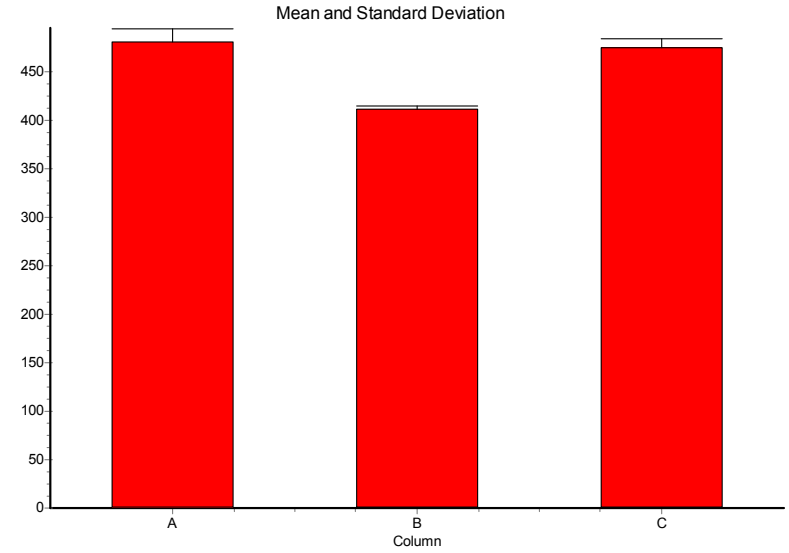

Figure 2: Weight of cauda epididymis in three groups of rats. Levels of significance values are (mean ± S.D.; n = 6), A: Weight. of c.epididymis of control group, B: Weight. of c.epididymis of treated group, C: Weight. of c, epididymis of recovery group.

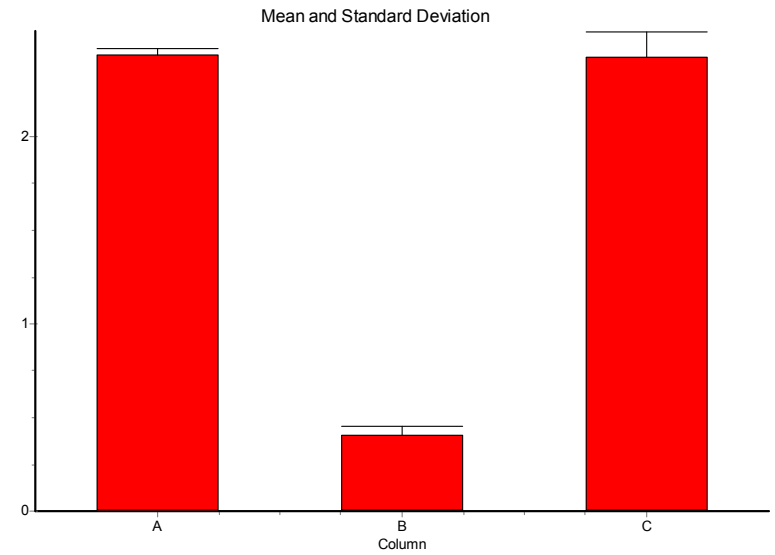

Fig.: 3-Sperm count in three groups of rats. Levels of significance values are (mean ± S.D.; n = 6) A: Sperm count in control group, B: Sperm count in treated group
C: Sperm count in recovery group.

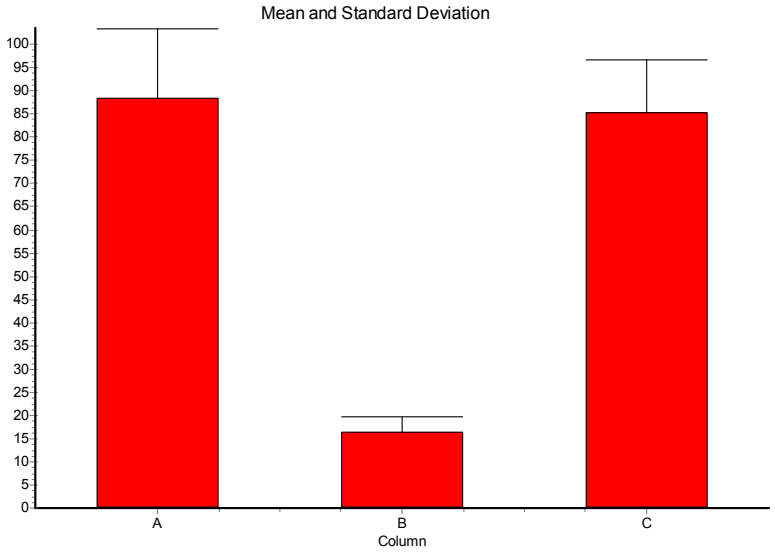

Fig.: 4- Testosterone in three groups of rats.A: Testosterone in control group,
B: Testosterone in treated group, C: Testosterone level in recovery group.

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..

Table 2: The relative blood cell counts-

Groups	Control (n=6)	Treated (n=6)	Recovery (n=6)
Variables	X ± S.D.) Minima-Maxima	(X ± S.D.) Minima-Maxima	(X ± S.D.) Minima-Maxima
RBCs x 10^6/mm^3	8.22 ± 0.60 (7.38- 8.78)	6.05 ± 0.57 (5.20- 6.62)	8.2 0± 0.26 (7.86-8.48)
WBCs x 10^3/mm^3	6.81 ± 0.49 (6.00- 7.40)	5.86 ± 0.33 (5.40-6.40)	6.83 ± 0.37 (6.18-7.24)
Platlets x 10^3/mm^3	645.0 ± 6.78 (636-654)	597.3 ± 14.02 (583- 616)	642.6 ± 16.18 (628- 664)
Lym. x 10^3/mm^3	5.58 ± 0.42 (4.80-5.90)	5.15 ± 0.30 (4.80-5.60)	5.41 ± 0.18 (5.20-5.70)
Mon. x 10^3/mm^3	1.06 ± 0.01 (1.05-1.08)	0.61 ± 0.14 (0.40-0.80)	1.37 ± 0.06 (0.94-1.10)
Gran.x 10^3/mm^3	2.93 ± 0.20 (2.68-3.22)	1.73 ± 0.41 (1.32-2.28)	2.93 ± 0.11 (2.80-3.08)
% Lymphocytes	58.22 ± 2.68 (53.45-60.66)	68.75 ± 4.00 (61.93-74.01)	57.94 ± 1.22 (56.11-59.34)
% Monocytes	11.11 ± 0.42 (10.63-11.80)	8.26 ± 2.01 (4.83-10.30)	10.95 ± 0.57 (10.15-11.54)
% Granulocytes	30.65 ± 2.43 (28.03-34.74)	22.96 ± 4.23 (18.80-29.03)	31.08 ± 1.23 (30.04-32.82)

In Table 2 mostly all the blood cell counts are decreasing. But this reduction is not significant.

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)-

Table 3: The relative blood parameters-

Groups	Control (n=6)	Treated (n=6)	Recovery (n=6)
Variables	X ± S.D.) Minima-Maxima	(X ± S.D.) Minima-Maxima	(X ± S.D.) Minima-Maxima
HGB (g/dl)	13.26 ± 0.46 (12.60-13.80)	10.16 ± 0.46 (9.60-10.80)	13.03 ± 0.32 (12.70-13.60)
HCT %	48 ± 1.18 (46.60-49.80)	41.25 ± 1.08 (39.80-42.40)	47.57± 1.23 (46.20-49.05)
MCV (fl)	58.20 ± 0.91 (57.20-59.40)	53.15 ± 0.55 (52.40-53.80)	58.41 ± 0.62 (57.72-59.20)
MCH (pg)	16.30 ± 0.64 (15.40-17.00)	16.58 ± 0.19 (16.30-16.80)	15.62 ± 0.59 (15.20-16.80)
MCHC(g/dl)	28.10± 0.69 (27.00-28.80)	31.26 ± 0.43 (30.80-31.90)	28.58 ± 0.64 (27.50-29.30)

RDW (%)	15.58 ± 0.40 (15.00-16.00)	16.53± 0.62 (15.80-17.40)	15.33± 0.87 (14.26-16.41)
MPV (fl)	10.70 ± 0.54 (9.80-11.40)	10.53 ± 0.43 (10.00-11.20)	9.81 ± 0.68 (8.80-10.80)
PDW (%)	15.65± 0.41 (15.00-16.20)	15.66± 0.43 (15.00-16.20)	15.46± 0.78 (14.57-16.52)
PCT (%)	0.3166 ± 0.01 (0.28-0.33)	0.4298± 0.00 (0.42-0.43)	0.3155 ± 0.01 (0.30-0.32)

Slight increase in- MCH (pg), MCHC (g/dl), RDW (%), PDW (%).
Slight decrease in- HGB (g/dl), HCT %, MCV (fl), MPV (fl).
Major increase in- PCT (%).

Liver function enzymes-Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) Alkalyne phosphatase (Alk.PO_4), Serum total protein (TP) were also studied

Table 4—The relative liver function enzymes-

Groups	Control (n=6)	Treated (n=6)	Recovery (n=6)
Variables	X ± S.D.) Minima-Maxima	(X ± S.D.) Minima-Maxima	(X ± S.D.) Minima-Maxima
SGOT (u/l)	171.00 ± 6.78 (162-180)	299.33 ± 8.64 (288-310)	169.83± 4.95 (165-178)
SGPT(u/l)	154.33± 2.94 (150-158)	127.00 ± 3.16 (123-132)	153.66 ± 4.63 (148-160)
Alk. PO_4 (u/l)	159.33± 4.84 (152-166)	77.83 ± 4.84 (73-84)	156.83± 5.26 (149-163)
TP (g/dl)	8.56± 0.37 (8.10-9.10)	6.21 ± 0.28 (5.80-6.60)	8.25 ± 0.62 (7.50-9.20)

Increase in- SGOT (u/l).
Decrease in- SGPT (u/l), Alk. PO_4 (u/l), TP (g/dl).

Discussion

The epididymis is a site which can be exploited for male contraception without undue side effects. It has been identified as the site where the essential post testicular sperm maturation and storage occurs. Nevertheless, little is known about the process of sperm maturation and factors affecting it[22] .Recently many laboratories are engaged in developing a male contraceptive from plants. .[23] Some causes that are responsible for low testosterone levels, include congenital problems such as aging, chronic illness, drugs, starvation, stress, head trauma, infections, cancers, surgeries, alcoholoism, removal or trauma of the testicles and infection or twisting of the testicles in their sack [24] .Many people are now relying on herbal medicines for health care [25] .The results showed that hematological parameters, were normal after treatment with the plant.

Increase in percentage of sperm abnormalities have most frequently been observed as one of the earliest indicators of testicular pathology. [26] Wistar rats have been reported to attain full spermatogenesis between the ages of 49-63 days. [27] This extract also possesses a reversible contraceptive action like report of others who have documented significant reduction in testosterone level [28]. Testosterone, qualitatively and quantitatively, is the most important androgen secreted by the tetis.It is responsible for the development of male characteristics during fetal life and during puberty and for the maintenance of virility in adulthood. The decreased testosterone level may also adversely affect the gametogenesis thereby decreasing the number of sperms [29]. In the present study, the reduced testicular and accessory sex organ weights indicate a wide spread damage [30] which could be due to reduced protein contents in these organs. Similar results have been observed With Semecarpus anacardium fruit. [31] and Mimosa pudica [32] .The epididymal maturation process is essential for sperm function. [33] Studies involving hypophysectomy, castration and androgen replacement therapy revealed that androgens are essential for physiological maturation and survival of the spermatozoa in the epididymis [34.]

As shown in Table 1, only the differences obtained from Mean values of weight of testes, cauda epididymis, testosterone level and sperm count were significant.

In Table 2 mostly all the blood cell counts are decreasing. But this reduction is not significant.

In Table 3- Slight increase in- MCH (pg), MCHC (g/dl), RDW (%), PDW (%).

Slight decrease in- HGB (g/dl), HCT %, MCV (fl), MPV (fl).

Major increase in- PCT (%).

In Table 4- Increase in- SGOT (u/l).

Decrease in- SGPT (u/l), Alk. PO_4 (u/l), TP (g/dl).

Conclusions

The present study revealed that the oil extract of seeds of Abrus precatorius produced reversible antifertility effects. The absolute whole body weights did not show significant alteration while weights of testis and cauda epididymis showed a significant reduction. A reduction in total protein in epididymal fluid on treatment of oil extract seeds of Abrus precatorius is also recorded. None of the hematological indices like hemoglobin content, blood cell counts (RBC and WBC) or other parameters revealed significant changes due to the treatment revealing the non-toxic nature of the extract. But the serum testosterone level registered a significant reduction, which is a probable cause for testicular and epididymal function as a result of androgen deprived effect. Hence sperm production and maturation process in both respective organs were affected by the extract.

References

1. Beckman LJ Harvey SM, factors affecting the consistent use of barrier methods of contraception. Obstet Gynecol 88 (suppl):655-715, **1996.**

2. Moore PJ, Adler NE, kegeles SM.Adolescents & the contraceptive pill: the impact of beliefs on intentions & use. Obstet Gynecal 88 (suppl):485-565, **1996**.

3. Cooper TG .epididymis .In: Neill JD, Knobil E, eds .Encyclopedia of reproduction 2.san Diego: Academic Press, 1-17, **1998.**

4. Orgebin -Crist M-C, Danzo BJ, Devies J.Endocrine control of the development & maintenance of sperm fertilizing ability in the Epididymis In: Hamilton DW, Greep RO, eds.Handbook of Physiology Washington DC: American Physiological Society, 319-338, **1975.**
5. Oyeyemi, M.O, olewatoy in, O., Ajala Leigh,O.O,Adesiji ,T,Fisayo ,The Spermiogran of male wistar rats treated aq. leaf extract Vernonia amygdalina Folia Veterinaria S2,2:98-101,**2008.**
6.Gupta RS ,Sharma R,Sharma A Bhatnager AK,Dobhal MP ,Joshi YC,etc.al.Effect of Alstonia Scholaris bark extract on testicular function on Wistar rats .Asian J.Androl:175 - 8, **2002**.
7. Lohia NK, Mani vannan B, Mishra PK, Pathak N., Sriram S, Bhande SS, et al. Chloroform extract of Carica papaya seeds induces long term reversible azoospermia in langur monkey Asia **2008.**
8. Udoh P, Kehinde A. studies on antifertility effect of pawpaw seeds (carica papaya) on the gonads of male albino rat. Phylother Res 13:226-8, **1999.**
9. Seetharam YN,sujeeth H,Jyothish waran G,Barad A,Sharanabasappa G,Umareddy B,et.al. Antifertility effect of ethanolic extract Amalakyadi churna in male albino mice Asian J Androl.
10. WHO (world Health Organization): WHO Bullet 602:199, **1982**.
11. Ratnasooriya W.D, Amarasekera A.S., Perera, N.S, Premakumara, G.A: J, Ethnopharmacol, 33:85-90, **1991.**
12. Kamboj V.P. A review of Indian medicinal plants with interceptive activity .Indian J med Res 87:336-355, **1988.**
12. Kamboj VP review of Indian medicinal plants with interceptive activity Indian J Mad Res 87:336-355, **1988.**
13. Bhargava SK, Effects of plumbagin on reproduction functions in male dog. Indian J Exp Biol 22:153-156, **1984**.
14. Madhusudhana Reddy C, Rama Krishna Murthy D, Saraswati B Patil .Antispermatogenic and androgenic activities of various extracts of Hibiscus rosasinesis in albino mice. Indian J Exp Biol 35:1170-1174**, 1987.**
15. K.sandhyakumany, R.G. Booby & M.Indira. Antifertility effect of Ricinus Commumis (Linn) on rats phytotherapy Research Phytother. Res .17.508-511, **2003.**
16. R.D.Pokharkar, R.K.Saraswat, Sheetal Kotkar, Survey of plants having antifertility activity from western ghat area of Maharastra state, Indian Journal of Herbal and Toxicology, **2009**.
17. WWW.ayurvdakalamandiram.com/herbs.htm.
18. Fox R.R: The rabbit as research subject. The physiol 27,330-346, **1984.**
19. Gay W.The dog as research subject. The physiol .27, 133-141, **1984.**
20. Gill 111, T.J: The cat as research subject The Physiol 28, 9-17, **1985.**
21. Jonas A.M: The mouse as in biomedical research .The physiol .27, 177-189, **1984.**
22 Mohammad Hossein Dehghan,Thomus Martin ,Robabeh Dehghanan Antifertility effect of Iranian neem seed alcoholic extract on epididyamal sperm of mice, Iranian Journal of reproductive medicine Vol.3.no2 pp:83-89,**2005.**
23. Jenson J.T .male contraceptive .Curr women's Health Rep.2 (5):338-45, **2002.**

24.Walid H.EL Tantawy ,Abeer Temraz, Omayma D.El-Gindi ,Free Serum Testosterone level in male rats treated with Tribulus alatus extract ,International Braz J urol val.33(4)554-559,July August, **2007.**
25. Tyler VE: A text book of Herbs of choice: The Therapeutic use of Phytomedicinals Norwood, Haworth Pr Inc.P.I **1994.**
26. Noakes ,DE ,T.J Parkinson and G.C.W. England Arthurs Veterinary Reproduction and Obstetria 8th edition Saunders Edin burgh,pp:695-751.isbn:o-7020-255-9,**2001.**
27.swerdloff ,R.S.,Walsh P.C,Jacobs H.S & Odell,W.D serum L.H & FSH during sexual maturation in the rat :effect of castration and cryptochidism.Endocrinolaogy 88 ,120-128,**1971.**
28. Pokharkar R.D., Saraswat R.K, Pokharkar M. R., Contraceptive efficacy of Butea monosperma seed extract in wistar rat, Pharmacologyonline 2:722-726, **2009**.
29. G.unnikrishnan R.shivabalan, J.Lillian, Subacute Toxicity of fluoxetine hydrochloride on fertility of male Wistar rats, Journal of Herbal medicine and Toxicology 3(1) 23-30, **2009.**
30.Keel BA ,Abney To influence of bilateral cryptorchidism in the mature rat :attraction in testicular function & serum hormone level Endocrinology 107;126-33,**1980.**
31. Sharma A, Verma P K, Dixit V.P Effect of semecarpus anacardium fruits on reproductive function of male albino rat's .Asian J Androl S: 121-4, **2003.**
32. Pokharkar Raghunath D., Saraswat Rajeshwari K., Kanawade Minal G., Contraceptive evaluation of oil extract of Mimosa pudica (L.) in wistar rats, Pharmacologyonline, **2009.**
33. Nieschlag E & Habenicht U.F Spermatogenesis Fertilization contraception Molecular, cellular and Endocrine events in male reproduction .springer-Verlag, Berlin **1992.**
34 .Kastner D, Apfelbach R.effect of cyproterone acetate on mating behavior, testicular morphology, testosterone level & body temperature in male ferrets in comparison with normal & castrated males. Hoin ref.25:178-184, **1987**.