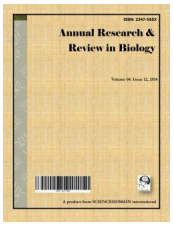

Annual Research & Review in Biology

21(4): 1-8, 2017; Article no.ARRB.38608
ISSN: 2347-565X, NLM ID: 101632869

Effect of Chronic Oral Administration of *Ruta montana* L. Areal Part Extract on Fertility Potential in *Albino* Rats

Mounira Merghem[1*], Saliha Dahamna[1], Kaoula Samira[2] and Seddik Khennouf[1]

[1]Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature Life Sciences, Ferhat Abbas University Setif-1, 19000 Setif, Algeria.
[2]CHU University Hospital Saadna Mohamed Abdenour, Sétif, Algeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author MM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SD and SK managed the literature searches. Author KS examined the histological sections. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/38608
Editor(s):
(1) Xiao-Xin Yan, Professor, Department of Anatomy & Neurobiology, Central South University Xiangya School of Medicine (CSU-XYSM), Changsha, China
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Hanan E.-S. Ali, Egyptian Petroleum Research Institute, Egypt.
(2) Daohong Chen, China.
(3) Stalin Chandrasekeran, Sunrise university, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/22474

Original Research Article

Received 2nd December 2017
Accepted 20th December 2017
Published 27th December 2017

ABSTRACT

Aims: The objective of the present study is to evaluate the effect of the aqueous extract of *Ruta montana* L. on the fertility in adult male and female rats.
Methodology: Forty healthy adult rats of both sexes were divided into 4 Groups of 10 rats each. During the treatment period, 100, 300 and 600 mg/kg/day of the extract were orally administered to Groups I, II and III respectively, while the control group received distilled water and served as control. The daily administration was carried out for a period of 90 days.
Results: The results did not show any significant change in ovaries weight. However, a significant decrease in testis, epididymis, and seminal vesicles weights was detected, as well as a reduction in

**Corresponding author: E-mail: mouniramerghem@yahoo.fr;*

the number and the motility of spermatozoids in rats treated with the doses of 300 and 600 mg/kg.
Conclusion: A dose of 300 mg/kg and 600 mg/kg of aqueous extract of *Ruta Montana* L. was able to cause a decrease in sexual organs weight of male rats and in the number and the motility of spermatozoids.

Keywords: Ruta montana L.; fertility; sperm motility; sperm counts; histological alterations.

1. INTRODUCTION

The use of plants in the management of diseases has been reported since antiquity, and has continuously grown over time. Various medicinal plant extracts have been tested for their anti-fertility activity in both males and females. Some of these plants had spermicidal effects, other caused reduction in the sperm counts, altered the mobility of the sperm, caused testicular change and altered hormone levels [1].

The medicinal plant *Ruta montana* L. (Rutaceae), is known in Algeria as “mountain rue” or “Fidjel” [2]. This plant is used in folk medicine as an antioxidant [3], hypoglycemic [4], antirhematic [5], antihelmintic, antiepileptic and antipyretic. It is also used in the treatment of intestinal disorders, hepatic diseases [6], and vitiligo [7]. *Ruta montana* L. contains various active principles which are able to inhibit the growth of mycobacteria [8]. In Algerian folk medicine, *Ruta montana* is used against child fevers and as an abortive drug [9]. To our knowledge no studies were conducted and published on the effect of this plant on fertility parameters in male and female rats. In the present study extract of *Ruta Montana* was tested to investigate a possible effect of this plant on Fertility potential in Albino rats.

2. MATERIALS AND METHODS

2.1 Plant

Ruta montana L. was collected in October, from Beni aziz region, in the North-East of Algeria, it was identified by Pr. Laouar H. (Department of Ecology & Plant Biology, Faculty of Nature and Life Sciences, university Ferhat Abbas, Sétif 1). A voucher specimen was kept at the herbarium of the laboratory of botany of the same faculty.

2.2 Animal

Male and female *Albino Wistar* rats were used in this study. The rats were purchased from Pasteur Institute (Algiers, Algeria). These animals were kept in the animal house, at a temperature of 20°C and under a natural photoperiod cycle. The animals were housed in plastic cages (5 rats per cage) and had free access to standard commercial diet and tap water.

2.3 Preparation of Plant Extract

The aerial parts of *Ruta montana* L. were cleaned, dried in shade for 2 weeks at room temperature and ground into fine powder using an electric grinder. The aqueous extract was prepared according to the method described by [10], with some modifications. Briefly, 100 g of *Ruta montana* L. powder was mixed with 1000 mL of boiled distilled water (100°C) at room temperature during 72 h. The mixture was filtered using Wattman filter paper n°3 and then evaporated in rotary vacuum evaporator and the resulting extract was stored at -4°C until use.

2.4 Experimental Design

Albino Wistar rats of both sexes, weighting between 150 and 200 g, were divided into four groups of 10 rats each (5 females and 5 males):

Control group: Rats received normal saline solution daily for 90 days by oral route.

- **Group I:** Received aqueous extract of *Ruta montana* L. at the dose of 100 mg/kg of body weight, daily for 90 days.
- **Group II:** Received aqueous extract of *Ruta montana* L. at the dose of 300 mg/kg of body weight, daily for 90 days.
- **Group III:** Received aqueous extract of *Ruta montana* L. at the dose of 600 mg/kg of body weight, daily for 90 days.

At the end of the treatment period, animals were fasted overnight with free access to water ad libitum. They were subsequently anesthetized with diethyl ether and sacrificed. After that, testes, epididymis, seminal vesicles and ovaries

were dissected out, washed with saline solution then fixed in 10% formalin for histopathological examination.

2.5 Organ's Weight

After the sacrifice of animals, testes, epididymis, seminal vesicles and ovaries were carefully removed and weighed individually (absolute organ weight). Organ weights were expressed in mg/100 g of body weight.

2.6 Sperm Suspension

The epididymis of each rat was placed in 1 mL of ringer buffer. The spermatozoa were obtained by making small cuts in caudal epididymis and diluted by adding 9 mL of ringer buffer (1/10) and then incubated at 37°C for 10 minutes. This suspension was used to evaluate the motility and counts of sperm.

2.7 Sperm Count and Motility

A sample of sperm suspension was taken and the number of sperm was counted using a haemocytometer under the light microscope. Four squares were counted in triplicate. The count was expressed as 2500/mm of suspension. Sperm motility (%) was also assessed immediately by counting both motile and immotile spermatozoa and calculated by the following formula:

> Number of motile sperm x 100/total number of motile and immotile sperm.

2.8 Histopathological Examination

Organs of treated and control rats were taken out. They were weighed and examined for gross lesions. Similar samples were fixed in 10 % formalin solution, dehydrated in graded (70-90 %) alcohol, cleared in xylene, and placed and embedded in paraffin wax. To perform the histology of tissues, 5-6 µm sections were prepared using Microtome (Leica, RM 2145). Then these sections deparaffinated in xylene, passed through 70% to 90% alcohol, and stained with hematoxylin and eosin (H&E). The slides prepared by this process were observed under light microscopy [11].

2.9 Statistical Analysis

The results are expressed as the mean value ± standard deviation. One-way analysis of variance (ANOVA) followed by the Tukey test was performed to assess differences between groups. Differences were considered significant at $p < 0.05$. Statistical analyses were performed with the software GraphPad Prism 5®.

3. RESULTS AND DISCUSSION

Several lines have suggested that some herbal drugs can be used as anti-fertility agents in mice, rats, rabbits and even humans [12]. Although many reports have showed that the use of plant extract can affect reproductive physiology of the animal.

In the present study, and in order to evaluate the effect of the chronic treatment of the aqueous extract of *Ruta Montana* L. on the fertility of both sexes, 4 groups of rats were used: a control group and 3 treated groups (100, 300, 600 mg/kg).

3.1 Effect of *Ruta montana* L. Aqueous Extract on Rat's Organ Weight

Organ's weight is an important index of physiological and pathological status in human and animals. Absolute and relative organ weights of 90 days-treated rats are shown in Table 1. The administration of the aqueous extract of *Ruta montana* L. did not cause any statistical difference in ovaries weight. Whereas, the treatment with the doses 300 and 600 mg/kg caused a significant decrease in testis, epididymis and seminal vesicles weight, when compared to the control group. Moreover, gross examination of internal organs of all the rat's revealed no detectable abnormalities.

The obtained results showed a significant weight reduction in testes, epididymis and seminal vesicles at the doses 300 and 600 mg/kg when compared to the control group (Table 1). This weight reduction was dose dependent. Generally, reductions in internal organ weight are simple and sensitive indices of toxicity after exposure to toxic substances [13]. The significant reduction of testis weight is known to be mostly related to the number of spermatids and spermatozoa present in the tissue [14]. Such reduction in the weight of reproductive organs is indirectly supports the reduced availability of androgen [15]. It is known that the accessory sex organs are androgen-dependent target organs and manifest differential sensibility to androgens for maintenance of their structure and function. It

is also known that, any change in circulating androgens would affect the internal microenvironment of epididymis and thereby lead to alteration in sperm motility and metabolism [16]. Androgen deprivation not only suppresses spermatogenesis, leading to low sperm concentration, but also alters the epididymal milieu which renders it hostile for maturation and survival of the spermatozoa [17,18]. Testosterone, an important androgen, plays a pivotal role in maturation, spermatogenesis and the maintenance of accessory sex organs [19]. The structural and functional integrity of reproductive tissues depends on the circulating androgen [20] and therefore, any small change in testosterone content may result in reductions in weights of reproductive organs.

3.2 Effect on Sperm Count and Motility

The results of the sperm count and motility were presented in Table 2. It was clear that animals treated by the aqueous extract of *Ruta montana* L. (100, 300, 600 mg/kg) showed evidence of a dose-dependent toxicity on epididymidal sperm parameters. The sperm count and motility were significantly decreased in group II and group III when compared to the control one.

Sperm characteristics are important reproductive indices as they account for male fecundity. The aqueous extract at the doses of 300 and 600 mg/kg produced a significant reduction in the sperm count and motility. Two possible hypotheses may be proposed to explain this reduction. One hypothesis is that the principle active of the extract may alter the pituitary gonadotropins hormones: luteinizing hormone (LH) and follicle stimulating hormone (FSH) [21]. It is well known that the weight, size and the secretary function of testes, epididymis and seminal vesicles are closely regulated by androgens hormones [22]. The production of the sperm cells (spermatozoa) and testosterone in testis are mainly regulated by the follicle stimulating hormone and Luteinizing hormone, which are released from the interior pituitary [23]. FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in leydig cells of testis [24]. Low levels of these hormones decrease endogenous testosterone secretion from the testis depriving developing sperm of the signal required for normal maturation and also it suppress testicular steroidogenesis and spermatogenesis [21] since the pituitary-testicular axis is a central regulatory conduit for testicular function that culminates in the production of spermatozoa [25]. Besides hormonal alteration, the alternative hypothesis is that the principles active may induce oxidative stress in testicular tissue and stored germ cells leading to generation of free radical products, as they exert a detrimental effect on spermatogenesis [26].

3.3 Effect of *Ruta montana* L. Aqueous Extract on Organ's Histology

Histological examination of testes of control group showed normal histological structure of seminiferous tubules associated with complete spermatogenic series as demonstrated in Fig. (1C). Testes of rats given 100 mg/kg of *Ruta montana* L. aqueous extract had similar

Table 1. Effect of chronic oral administration of aqueous extract of *Ruta montana* L. on rats organs weight

	Relative organ weight			
	Control	**Group I (100 mg/kg)**	**Group II (300 mg/kg)**	**Group III (600 mg/kg)**
Testis	0,58±0,07	0,48±0,13	0,23±0,15*	0,19±0,10**
Epididymis	0,22±0,00	0,19±0,04	0,11±0,02**	0,07±0,00**
Seminal vesicle	0,90±0,43	0,55±0,26	0,13±0,05*	0,16±0,11*
Ovaries	0,04±0,01	0,04±0,00	0,03±0,00	0,04±0,007

*Values represent the mean ± SD (n =5). * p<0.05; ** p<0.01*

Table 2. Effect of *Ruta montana* L. aqueous extract on sperm count and motility

	Control	**Group I (100 mg/kg)**	**Group II (300 mg/kg)**	**Group III (600 mg/kg)**
Count (2500/mm^3)	13.21±1.99	9.66±2.22	5.4±0.04**	1±0.00***
Motility (%)	63.73±2.25	60.05±2.08	19.42±4.30***	0.00±0.00***

*Values are presented as means ± SD (n=5); ** P < 0.01, *** P < 0.001 compared with control*

histological appearance as the control rats (Fig. 1 D1) with active spermatogenesis. However, testes of rats of group II and III (treated with 300 and 600 mg/kg of *Ruta montana* L.) revealed degeneration of seminiferous tubules with absence of sperm in tubular lumen as shown in Fig. (2 D2) and Fig. (2 D3).

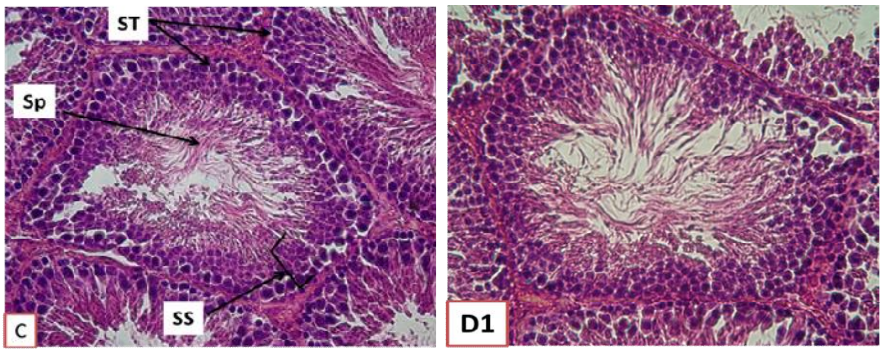

Fig. 1. Histological sections of testes of control group (C) and group I (D1) treated with 100 mg/kg of *Ruta montana* L. aqueous extract after 90 days of treatment (HE X 200)

Showing normal histological structure of semiferous tubules associated with complete spermatogenic series and spermatozoa in the lumen. ST: Seminiferous tubules; Sp: Sperm; SS: Stages of spermatogenesis

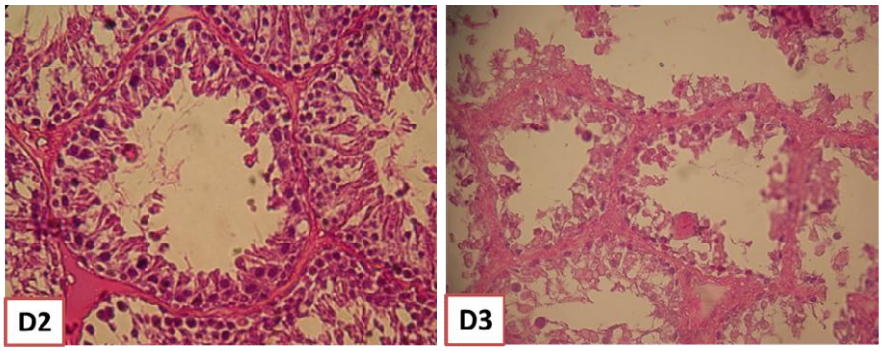

Fig. 2. Histological sections of testes of group II (D2) and group III (D3) treated with 300 mg/kg and 600 mg/kg respectively of *Ruta montana* L. aqueous extract after 90 days of treatment (HE X 200)

Showing degeneration of seminiferous tubules with absence of sperm in tubular lumen

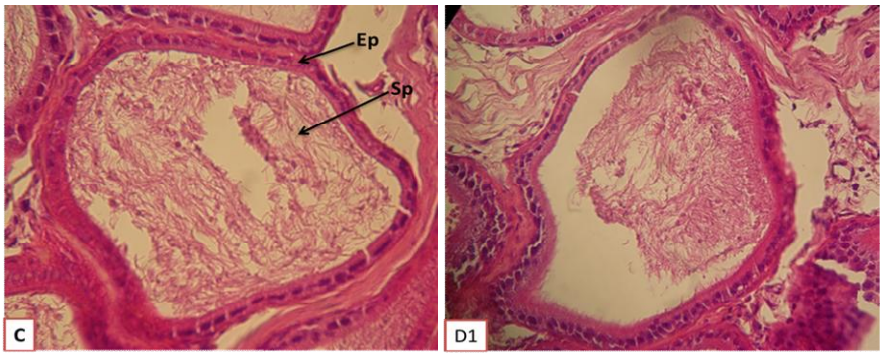

Fig. 3. Histological sections of the epididymis of control group (C) and group I (D1) treated with 100 mg/kg of *Ruta montana* L. aqueous extract after 90 days of treatment (HE X 200)

Showing normal tubules that contained spermatozoa in the lumen. Ep: Epethelial cells; Sp: Sperm

Epididymis of the control group and the group I (100 mg/kg) showed normal tubules with spermatozoa in the lumen (Fig. 3C and D1). Whereas, the treatment with higher doses (300 and 600 mg/kg) caused several damages in epididymis tubules with absence of sperm (Fig. 4 D2 and D3). On the other hand, no histological changes were observed in ovaries of females of all treated groups when compared to the control group (Figs. 5 and 6).

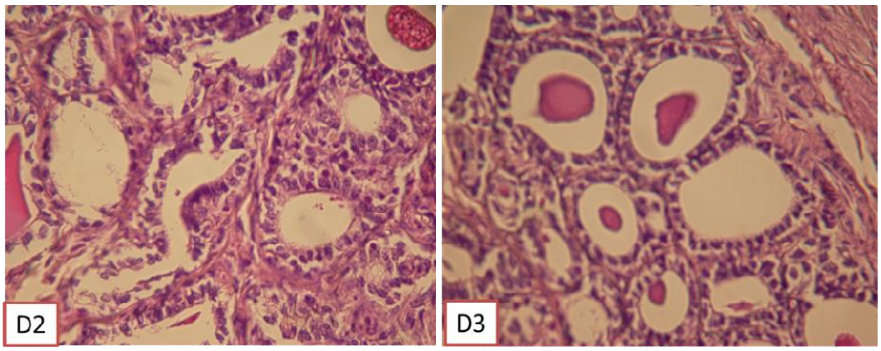

Fig. 4. Histological sections of the epididymis of group 2 (D2) and group 3 (D3) treated with 300 mg/kg and 600 mg/kg respectively of *Ruta montana* L. aqueous extract after 90 days of treatment (HE X 200)

Showing damage in epididymal tubules and absence of sperm

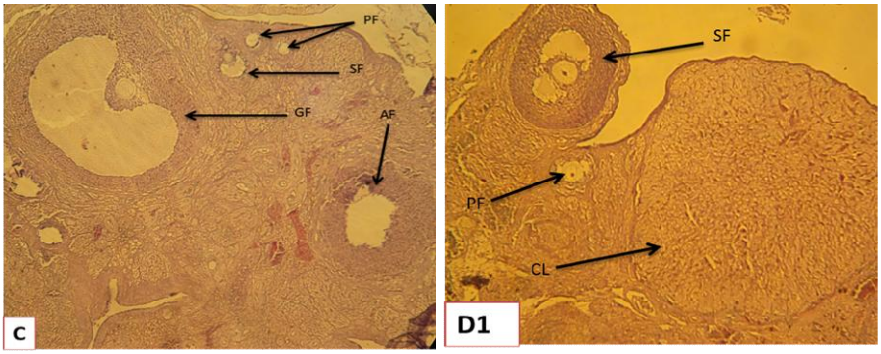

Fig. 5. Histological sections of Ovary of control group (C) and group I (D1) treated with 100 mg/kg of *Ruta montana* L. aqueous extract after 90 days of treatment (HE X 200)

Showing normal structure. PF: Primary follicle; SF: Secondary follicle; GF: Grafian follicle; AF: Atresia follicle; CL: Corpus luteum

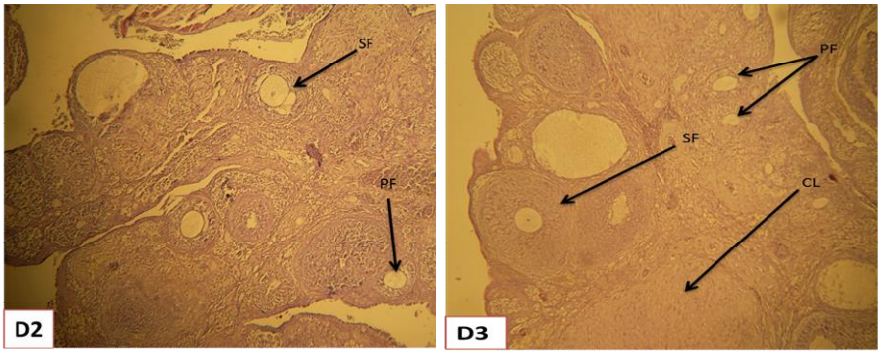

Fig. 6. Histological sections of Ovaries of group II (D2) and group III (D3) treated with 300 mg/kg and 600 mg/kg respectively of *Ruta montana* L. aqueous extract after 90 days of treatment (HE X 200)

Showing normal structure. PF: Primary follicle; SF: Secondary follicle; CL: Corpus luteum

4. CONCLUSION

It was concluded that the oral administration of aqueous extract of *Ruta montana* L. in rats at 100 mg/kg did not caused any change in weight of reproductive organs . However, the doses of 300 and 600 mg/kg caused a decrease in testis, epididymis, and seminal vesicles weights and reduction in the number and the motility of spermatozoids.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alagammal M, Sakthidevi G, Mohan VR. Anti-fertility activity of whole plant extracts of *Polygala rosmarinifolia* Wight & Arn against male albino rats. Journal of Advanced Pharmaceutical Sciences. 2013; 385-393.
2. Bellakhdar J. La pharmacopée marocaine traditionnelle. Maroc: Ed Ibis Press; 1977.
3. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. Food Chemistry. 2006;97:654–660.
4. Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, Benjelloun W. Phytotherapy of hypertension and diabetes in oriental Morocco. J Ethnopharmacol. 1997;58:45–54.
5. Boukef K. Les pratiques parallèles de soins: Aspects sociologiques et éthiques Comité National D'éthique Médicale. 13eme Conférence Annuelle, Dec 4; Sousse, Tunisie; 2009.
6. Bnouham M, Mekhfi H, Legssyer A, Ziyyat A. Ethnopharmacology Forum Medicinal plants used in the treatment of diabetes in Morocco. Int J Diabetes Metabolism. 2002; 10:33–50.
7. Milesi S, Massot B, Gontier E, Bourgaud F, Guckert A. *Ruta graveole* L.: A promising species for the production of furanocoumarins. Plant Science. 2001; 161:189–99.
8. Sqalli H, El ouarti A, Ennabili A, Ibnsouda S, Farah A, Haggoud A, Houari A, Iraqui M. Évaluation de l'effet antimycobactérien de plantes du centre-nord du Maroc. Bull. Soc.Pharm. Bordeaux. 2007;146:271-288.
9. Bellakhdar J, Claisse R, Fleurentin J, Younos C. Repertory of standard herbal drugs in the Moroccan pharmacopoeia. J Ethnopharmacol. 1991;35:123–143.
10. Mbiantcha M, Kamanyi A, Teponno R B, Tapondjou L A, Watcho P, Nguelefack T B. Analgesic and anti-inflammatory properties of extracts from the Bulbils of *Dioscorea bulbifera* L. var sativa (Dioscoreaceae) in Mice and Rats. Evidence-Based Complementary and Alternative Medicine. 2011;1-9.
11. Bensalem-Bendjelloul M. Techniques histologiques théorie et pratique. Office des publications universitaires. 1998;6-41.
12. Koneri R, Saraswati CD, Balaraman R, Ajeesha EA. Antiimplantation activity of the ethanolic root extract of *Momordica cymbalaria* Fenzl in rats. Indian Journal of Pharmacology. 2007;39:90-96.
13. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A and Khetani V. A 90- day oral gavage toxicity study of D-methylphenidate and D, Lmethylphenidatein Sprague-Dawley rats. Toxicology. 2002;179:183-196.
14. Gupta RS, Chaudhary R, Yadav RK, Verma SK, Dobhal MP. Effect of Saponins of *Albizia lebbeck* (L.) benth bark on the rerproductive system of male albino rats. J. Ethnopharmacol. 2005;96:31-36.
15. Zeherea MN, Reddy SP, Reddy SP, Ravindra and Saraswati BP. Antispermatogenic and androgenic activities of *Momordica charantia* (Karela) in Albino rats. J. Ethnopharmacol. 1998; 61:9-16.
16. Khan PK, Awasthy KS. Cytogenetic toxicity of Neem. Food Chem. Toxicol. 2003;41: 1325-1328.
17. Setty BS. Regulation of Epididymal function and sperm maturation- endocrine approach to fertility control in male. Endocrinology. 1979;74:100-117.
18. Rao MV. Effect of alcoholic extract of *Solanum xanthocarpum* seeds in adult male rats . Indian J. Exp. Biol. 1988;26:95-98.
19. Keel AB, Abney T. Influence of bilateral cryptorchidism in the mature rat; Alteration

in testicular function and serum hormonal level. Endocrinology. 1980;107:1226-1233.
20. Chinoy NJ, Sheth KM, Seethalakshmi L. Studies on reproductive physiology of animals with special reference to fertility control. Comp. Physiol. Ecol. 1982;7-325.
21. Kusemiju TO, Osinubi AA, Noronha CC, Okanlawon AO. Effect of *Carica papaya* bark extract on the testicular histology of male SD rats. Nig. Quat. Jour. of Hosp. Med. 2010;20(3):133-137.
22. Almasad MM, Qazan WSH, Daradka H. Reproductive toxic effects of Artemisia herba alba ingestion in female Sprague-Dawley rats. Pak J Biol Sci. 2007;10(18): 3158-3161.
23. Steinberger E. Hormonal control of mammalian Spermatogenesis. Physiology Review. 1971;5111:1-22.
24. Wannang NN, Jimam NS, Gyang SS, Bukar BB, Gotom S. Effects of *Cucumis metuliferus* E Mey. Ex Naud (Cucurbitaceae) fruit extract on some male reproductive parameters in adult rats. African Journal of Pharmacy and Pharmacology. 2008;2(3):048-051.
25. Cheng CY, Wong EW, Yan HH, Mruk DD. Regulation of spermatogenesis in the microenvironment of the seminiferous epithelium: New insights and advances. Mol. Cell Endocrinol. 2010;315(1–2):49–56.
26. Ghosh D, Das UB, Ghosh S, Mallick M, Debnath J. Testicular gametogenic and steroidogenic activities in cyclophasphamide treated rats: A correlative study with testicular oxidative stress. Drug Chem Tox. 2002;25:281-291.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22474