

Research Progress of Hibiscus Sabdariffa Medical Plant as Infertility Agents on Male Rabbits

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ABSTRACT

Medical plants are characterized by physiological active principles that have been utilized in traditional medicine years ago in treatment of different diseases. Previous studies indicated that *Hibiscus sabdariffa* has ethno-medical and ethno-veterinary characteristics; however, the relationship between and male reproductive hormones levels has not been clearly demonstrated. The objective of the current work was to examine the impact of 30% concentration *H. sabdariffa* extract on reproductive hormones and histological effects of the testis of adult male rabbits. Crude extract of blooms of *Hibiscus rosasinensis* has been demonstrated that there was definite antifertility effect of this extract in causing degenerative changes in the germinal epithelium of male rabbits. Effect of *H. sabdariffa* on basal levels of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) was studied using post pubertal healthy male rabbits. Calyx extract of *H. sabdariffa* at a dose of 300 mg/kg reduced reproductive hormones in experimental rabbits. In conclusion, calyx extract of *H. sabdariffa* can have negative impact on male reproductive hormones in rabbits and the histological formation of the testicle.

Keyword: *Hibiscus sabdariffa*, rabbits, Testosterone, FSH, LH

Introduction

Failure of conception is one of infertility parameters [1]. After successful ovulation, approximately 50% of conception failure could have associated to male factors [2]. Potential treatment using traditional medicine, for instance medicinal plants, as well as modern medicine has been studied [3, 4]. Assessment of medicinal plants as successful medications for such dysfunctions has become interested in both developed and developing countries [5]. Different studies revealed that several indigenous plants such as *Olmium Sandum*, *Piperlongus*, Cotton seed oil extract and Ayurvedic ingredients possess antifertility activity [6, 7]. Antifertility effect of *Hibiscus Sabdariffa* was previously examined in female mice [8]. In that study, *H. Sabdariffa* terminated the pregnancy in mice as a function of antiestrogenic and progesterone activity. *Hibiscus Sabdariffa* is a plant species belongs to genus *hibiscus*, family: *Malvaceae*, which is considered one of the most common flower plants worldwide including over than 300 species. Although *hibiscus* is a native to tropical Africa, it can also be found in other Southeast Asia countries. Nowadays, different extracts and molecules isolated from various plants are usually utilized in treatment of various causes of infertility in male. Such extracts include *Trichopuszeylanicus* ethanolic extract [9], *Vanda tessellata* flowers ethanolic extract [10], *Lepidium meyenii* lipidic extract [11], *Turneradiifuse* and *Pfaffiapaniculata* extracts [12], *Tribulusterrestris* extract [13], *Panaxginseng* roots [14] *Eurycomalongifolia* extract [15] *Terminalacatappa* seeds extract [16], *Rutachalepensis* aqueous extract [17], Polysaccharides of *Lycium barbarum* fruit extract [18], *Shengjingpill*; a Chinese formula of plant extracts [19], *Hibiscus macranthus* and *Basella alba* aqueous extracts [20], *Croton zambesicus* ethanolic extract [21], and *Astragalus membranaceus* and *Acanthopanax santicosia* aqueous extracts [22]. However, the antifertility activity of *hibiscus* flower has not been extensively examined. Therefore, the objective of the current work was to examine the impact of 30% concentration *H. sabdariffa* extract on reproductive hormones of adult male rabbits.

Materials and Methods

A total of 10 post pubertal healthy male rabbits weighting 3-3.5 Kg were used in this study. Study rabbits were kept in standard cages with maximum 10 rabbits per a cage, in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/ dark cycle according experimental protocols, and had free access to standard rabbits pellets and water throughout the experiment duration. Crude extract of plant flower was prepared by mixing 30 g of grinded flower with 100 mL of water. Prior to the treatment, blood samples were collected from marginal ear vein of each animal using sterile syringes and hypodermic needles for preliminary analysis of serum testosterone, LH, FSH hormones. Testosterone, FSH and LH measured using a commercial ELISA kit. Which is based on competitive binding of hormones on immobilised antibody. At the expiration day, i.e., 8 weeks later, blood samples were also collected. The hormone concentration estimate is repeated after the end of the treatment period. Statistical comparisons were made using the ANOVA test for comparison of data which obtained at the beginning of the experiment and after eight weeks. The results

expressed as mean $\pm$ S.E.M (standard error of means). Significant difference is written in different letters. Histology study : The male rabbits testes fixed in 10% formalin and embedded in paraffin then sections prepared at five-micron thick and stained with Hematoxylin and Eosin (H&E). The specimens examined under Olympus/3H light microscope-Japan.

Results

Hormones Level

The table 1 and figure 1 shows a significant decrease at ($p \leq 0.05$) in the concentration of the hormones in the blood serum of the male rabbits which treated with aqueous extract of hibiscus at a dose of (300 mg / kg) b.w daily for 8 weeks, compared to its value before the treatment. Results showed that the arithmetic mean of testosterone after treatment (0.69 ± 0.01), while its value was before treatment (1.4 ± 0.01), so is the same case with the Follicle Stimulating Hormone regarding a hormone, as the value of its arithmetic mean after the treatment (0.82 ± 0.01), while it was before the bitter treatment (1.53 ± 0.01), it did not differ with the luteinizing hormone, as the value of its arithmetic mean after the treatment reached (0.59 ± 0.01), while it was before the treatment (1.16 ± 0.01).

Table 1. Effect of *Hibiscus sabdariffa* calyx extract on circulating levels of selected male rabbits reproductive hormones

Hormones	Before treatment	After 8 weeks
Testosterone (nmol/L)	1.4 ± 0.01 A	0.6 ± 0.01 B
Follicle Stimulating Hormone (mIU/ml)	1.53 ± 0.01 A	0.82 ± 0.01 B
Luteinizing hormone (mIU/ml)	1.16 ± 0.01 A	0.59 ± 0.01 B

Values are expressed as mean $\pm$ SEM (n=10)

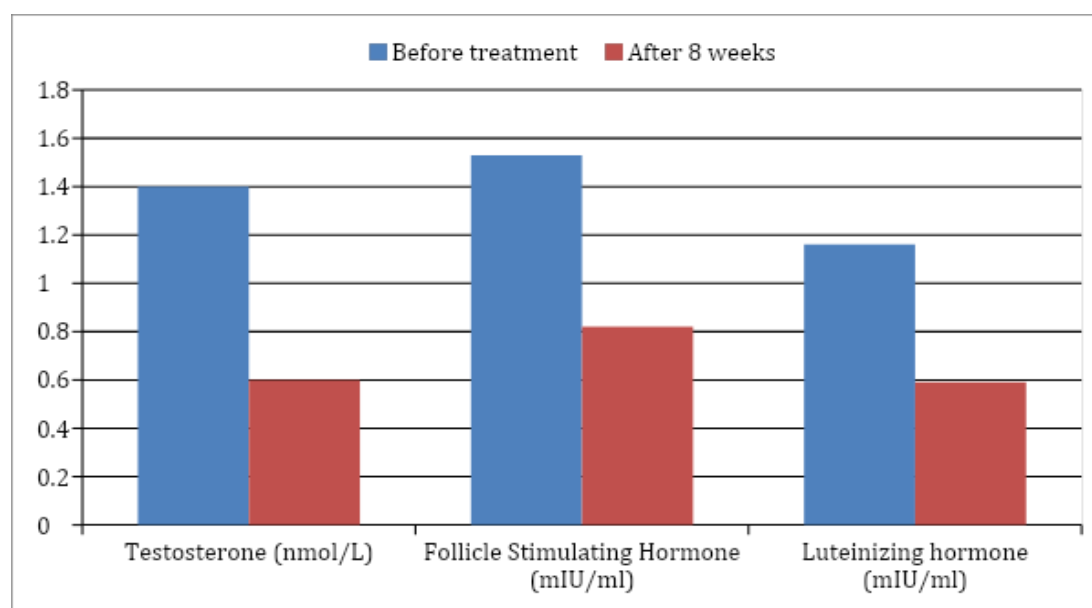


Fig1. Effect of *Hibiscus sabdariffa* calyx extract on circulating levels of selected male rabbits reproductive hormones

Results of the testicle histological study

Testes transverse section of male rabbits before treatment showing a normal seminiferous tubules collagen tissue spermatogonia cell. The microscopic examination of the testicles of rabbits showed the normal structure of the testis, as the seminiferous tubules appear in regular shapes and sizes as well as being lined with several rows of primary and secondary spermatogenic cells with the dominant cells (Sertoli cells) in addition to the containment of the cavities of the seminal tubules on many spermatids. (Fig.2). While transverse section of male rabbits Testes treated with *Hibiscus rosasinunsis* shows the dissolution of the connecting tissue between cells and the atrophy of the Leydig cells with congestion of some blood vessels between the seminal tubules and the irregularity of the cells that include the different stages of spermatogenesis, and the emptying of the seminal cavities of them . (Fig.3)

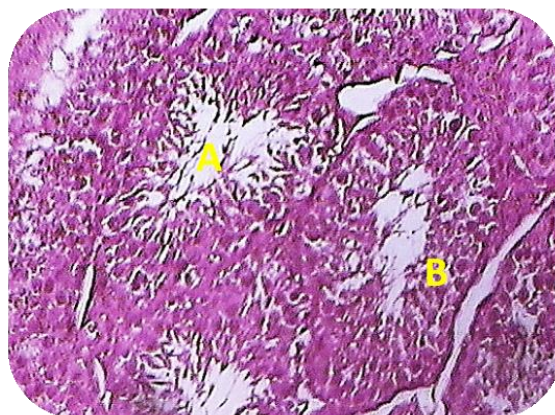


Fig.1 section of testes showing The normal structure of the testis ,The seminal tubules appear of regular shapes and sizes (A), as well as being lined with several rows of spermatogenic cells (B)

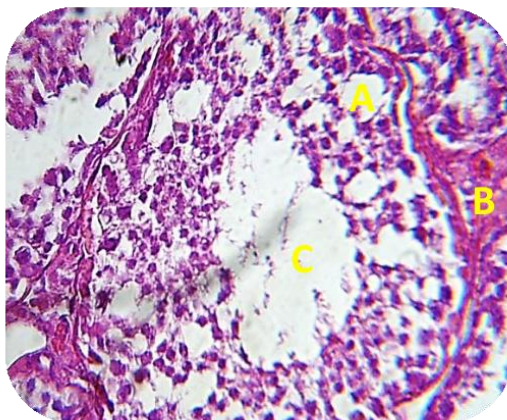


Fig.2 Section of testes showing demonstrates the histopathological changes represented by detachment of the connecting tissue between cells (A), congestion of some blood vessels between the tubules (B), irregularity of the lumen of the seminal tube and the absence of sperms (C).

Discussion

Adrenal cortex, gonads, and placenta are considered endocrine glands that secrete different steroidal hormones that produce their effect on target organs [23]. Testosterone is produced by the Leydig cells, and has hormonal and pro-hormonal characteristics, and is considered an essential hormone for the growth and functions of reproductive organs, particularly Sertoli cells that are responsible for the spermatogenesis [24]. On the other hand, in herbal medicine, health can be promoted and illness can be reduced as a function of natural substances in the plants [25], although medicinal plants might have negative consequences on public health and some physiological functions of the body [26]. In this study, treatment of study rabbits with *H. Sabdariffa* extract decreased the testosterone level. It has been indicated that treatment with plant materials reduced sperm count, motility, and viability as well as increased the amount of abnormal sperm [26], suggesting that the extract can cause an androgen depletion at the level of target, particularly caudal epididymis. Additional explanation for decrease of testosterone is that *H. Sabdariffa* extract contains protocatechuic acid, a simple phenolic compound

and biochemical marker of testicular toxicity [27, 28] that can suppress the testicular function. The decrease of testosterone hormone can further be established by connecting circulating FSH, as this hormone regulates the testicular development and function [29]. In rats, a dose of 4.6g/kg of *H. Sabdariffa* extract disintegrated sperm cells and induced testicular toxicity [26]. *H. subdariffa* has various compounds including: tannins, alkabidstriterpenoids, flavonoids, taraxeryl acetates Poly phenols, saponinshibisetin, cyandine glycosides, oxalates, and peroxidaes. Flavonoids have a variety of bioactivity including antifertility characterized by decrease of testosterone via loss the epididymis weight [30].

It is known that FSH is released by the pituitary gland. It can stimulate testicular growth and help producing vital protein to male fertility that aid in the creation of normal sperm cells and maintaining them until their release. The pituitary gland regulates the reproduction via the refined combined action of the FSH and LH on the gonads [31]. High levels of FSH in a male might mean damage of the testicles or they are not correctly functioning [32]. In the current study, however, FSH level has decreased, which is in line with previous report, which could be a function of flavonoids present in the extract [33, 34]. Flavonoids in the hibiscus extract with might cause hypothalamic and pituitary failure to secrete gonadotropin-releasing hormone GnRH, and failure of the pituitary to the synthesis of FSH and LH leading to ley dig cell failure to perform testosterone synthesis [34, 35]. Flavonoids are estrogenic compounds can occupy estrogen receptors inside the body [36] then cause decrease of FSH secretion and inhibit LH production, which is important for growth and control of the number of leydig cells and then decrease the secretion of testosterone from this cells [30, 37].

The results obtained are consistent with the results [38] which found that the obtained data in this study was revealed that spermatogenesis and sperm parameters significantly changes during treatment and was show adverse effect of extracts of Hibiscus rosasinensis on sperm parameter and histology of testis. The negative effect on fertility of this herb's aqueous extract is a study [39] Which was confirmed by conducting a clinical trial to prevent conceiving 21 women of one age group using the ethanolic extract of the hibiscus flower at a concentration of 750 mg per kg of body weight at a rate of 4 daily doses from the seventh day to the 22nd of the menstrual cycle, as the results confirmed preventing pregnancy for 14 of them for a period of four years. Accordingly, it was recommended to obtain a safe drug from hibiscus extract that is used as a natural contraceptive that has no side effects.

The presented study also agrees with a study of [40] that dealt with the level of hormones, sperm formation, and the histological effect of the aqueous extract of hibiscus at concentrations of 50 and 100 mg per kg body weight which was found to be that Hibiscus sabdariffa has adverse effect on spermatogenesis and sperm parameters of mice, And the same opinion with [41] which found that the weight of testis and epididymis showed a significant reduction and the fall in density of sperms and that of testosterone level are correlated to one another in treated animals with Hibiscus rosasinensis. [42], has described that antifertility action of Hibiscus rosasinensis is season dependent. He also reported that it causes significantly inhibitory action on spermatogenesis in mediated via pituitary gland without affecting pituitary adrenal and pituitary thyroid function in male albino rats.

Decreased spermatogenesis and its lack of presence in the lumen of the seminal tube may be due to a decrease in the level of the hormone testosterone responsible for the ripening of sperm, which has been transferred to the epididymis [43]. The atrophy of the Leydig cells can occur as the result of the decrease in LH hormone which important for growth and control of the number of leydig cells, and its inhibition causes a decrease of the secretion of testosterone [44].

Steroidogenesis leads to testosterone synthesis, this process take place in leydig cells. If the number or function of leydig cell is reduced, then testosterone production will be also reduced and once secrete, the testosterone is then bounded by androgen binding protein (ABP) secreted by sertoli cells, so when the testosterone is decreases the synthesis of ABP also decreases, causing decreased transport of testosterone to the epididymis which causes the atrophy of epithelial cells, Testicular Leydig cells, which reside in the testis interstitium, are the primary source of testosterone in males [45].

The research recommends studying more of the effects of aqueous extract on the reproductive organs, the possibility of using safe concentrations as a method of contraception, and studying the relationship between increased concentrations with the side effects of the extract.

Reference

- [1] WHO Laboratory Manual for the Examination and Processing of Human Semen.(2010). World Health Organization: Geneva, Switzerland, 5th ed.; 2010.
- [2] Shefi S, Turek JP (2006) Definition and current evaluation of subfertile men: review article. Intern Braz J Urol 32:385-97
- [3] Larsen U, Hollos M (2005) The importance of motherhood: a study of infertility in urban Northern Tanzania, 1-44. <http://db.jhuccp.org/ics-wpd/exec/icswppro.dll>

- [4] Feldman HR, Laura R (2004) The use of complementary and alternative medicine practices among Australian university students. *Complement Health Pract Rev* 9:173–9
- [5] Rates KMS (2001) Plants as source of drugs: review. *Toxicon* 39:603–13
- [6] Batta SK, and Santhakumari G .(1971). Antifertility effect of *olmiumsandum*& *Hibiscus-rosa-sinensis*. *Indian J. Med. Res.*, 59 (5): 777-81
- [7] Rajeshwan V, and Kamat RS, (2012). Review of Ayurvedic Drugs on Endocrine system *International J. of Health Science & Research*.2 (2).-
- [8] Pakrashi A, Bhattacharya K, Kabir SN, (1986). Flowers of *Hibiscus rosa-sinensis*, a potential source of contragestative agent. III: Interceptive effect of benzene extract in mouse. *Contracep.*, 34(5): 523-536
- [9] Subramoniam A, Madhavachandran V, Rajasekharan S, Pushpangadan A (1997) Aphrodisiac properties of *Trichopuszeylanicus* extract in male mice. *J Ethnopharmacol* 57:21–7
- [10] Kumar SKP, Subramoniam A, Pushpangadan P (2000) Aphrodisiac activity of *Vanda tessellata* (roxb.) Hook. Ex don extract in male mice. *Indian J Pharmacol* 32:300–4
- [11] Zheng BL, He K, Kim CH, et al (2000) Effect of a lipidic extract from *Lepidiummeyenii* on sexual behavior in mice and rats. *Urology* 55:598–602
- [12] Arletti R, Benelli A, Cavazzuti E, et al (1999) Stimulating property of *Turneradiffusa* and *Paffiapaniculata* extracts on the sexual behaviour of male rats. *Psychopharmacology* 143:15–9
- [13] Gauthaman K, Adaikan GP, Prasad VNR (2002) Aphrodisiac properties of *TribulusTerrestris* extract (Protodioscin) in normal and castrated rats. *Life Sci* 71:1385–96
- [14] Murphy LL, Cadena SR, Chávez D, Ferraro SJ (1998) Effect of American Ginseng (*Panaxquinquefolium*) on male copulatory behavior in the rat. *PhysiolBehav* 64(4):445–50
- [15] Ang HH, Ikeda S, Gan EK (2001) Evaluation of the potency activity of aphrodisiac in *Eurycomalongifolia* (Jack). *Phytother Res* 15:435–6
- [16] Ratnasooriya DW ,and Dharmasiri GM (2000) Effects of *Terminaliacatappa* seeds on sexual behaviour and fertility of male rats. *Asian J Androl* 2:213–9
- [17] Al-Qarawi AA (2005) Stimulatory effect of the aqueous extract of *Rutachalepensis* on the sex organs and hormones of male rats. *J Appl Res* 5:206–11
- [18] Luo Q, Li Z, Huang X, et al (2006) *Lyciumbarbarum* polysaccharides: protective effects against heat-induced damage of rat testes and H₂O₂-induced DNA damage in mouse testicular cells and beneficial effect on sexual behavior and reproductive function of hemi-castrated rats. *Life Sci* 79:613–21
- [19] Xu X, Yin H, Tang D, et al (2003) Application of traditional Chinese medicine in the treatment of infertility. *Hum Fertil* 6:161–8
- [20] Moundipa FP, Kamtchouing P, Koueta N, et al (1999) Effects of aqueous extracts of *Hibiscus macranthus* and *Basella alba* in mature rat testis function. *J Ethnopharmacol* 65:133–9
- [21] Ofusori DA, Oluwayinka OP, Adelakun AE, et al (2007) Evaluation of the effect of ethanolic extract of *Croton zambesicus* on the testes of Swiss albino mice. *Afr J Biotechnol* 6:2434–8
- [22] Liu J, Liang P, Yin C, et al. (2004) Effects of several Chinese herbal aqueous extracts on human sperm motility in vitro. *Andrologia* 36:78–83
- [23] Takahiro I. , Hirokuni S. , Naoyuki M. , Jumpei F. , Hidetomo I., Hiroshi Y. (2019). Analysis of Corticosterone and Testosterone Synthesis in Rat Salivary Gland Homogenates . *Front. Endocrinol.*, 17 July 2019 | <https://doi.org/10.3389/fendo.2019.00479>
- [24] Omotuyi I. , Ologundudu A., Onwubiko V., Wogu M., Obi F. (2010). *Hibiscus sabdariffa* Linn anthocyanins alter circulating reproductive hormones in rabbits (*Oryctolagus cuniculus*). *Journal of Diabetes and Endocrinology* Vol. 1(3), pp. 36-45, June 2010.
- [25] Craig, W.J. (1999). Health-promoting properties of common herbs. *Am J Clin Nutr.* 70(suppl): 491S-499S
- [26] Orisakwe OE, Husaini DC, Afonne OJ .(2004). Testicular effects of sub-chronic administration of *Hibiscus sabdariffa* calyx aqueous extract in rats. *Reprod Toxicol* 18: 295–298.
- [27] Dalziel JM.(1973). The useful plants of West Tropical Africa. London: Crown Agent for Overseas Government and Administrations Mill Bank; 1973. p. 129–30
- [28] Shen RS and Lee JP. (1994). Selected testicular enzymes as biochemical markers for procarbazine-induced testicular toxicity. *Arch Toxicol* 1994;55:233–8.

- [29] Olesen I A, Joensen U N, Petersen J H, et al.(2018). Decrease in semen quality and Leydig cell function in infertile men: a longitudinal study. *Hum Reprod.* 2018;33:1963-74. 15.
- [30] Carolin B. , Shinta N. , Sri N. , Salonia, A.(2019). Effects of Hibiscus rosa-sinensis Linn. Flower Extract on Epididymis, Prostate and Seminal Vesicles of Male Rats IBBJ Winter 2019, Vol 5, No 1
- [31] Daniele S., Pascale C., Eric R., Giorgia S., Giulia B., Livio C., Vincenzo R. and Manuela S.(2020). Follicle-Stimulating Hormone (FSH) Action on Spermatogenesis: A Focus on Physiological and Therapeutic Roles J. Clin. Med. 2020, 9, 1014; doi:10.3390/jcm9041014
- [32] Salonia, A.; Rastrelli, G.; Hackett, G.; Seminara, S.B.; Huhtaniemi, I.T.; Rey, R.A.; Hellstrom, W.J.G.; Palmert, M.R.; Corona, G.; Dohle, G.R.; et al.(2019). Paediatric and adult-onset male hypogonadism. *Nat. Rev. Dis. Primers* 2019, 5, 38.
- [33] Srivastav A, Chandra A, Singh M, et al. (2010). Inhibition of hyaluronidase activity of human and rat spermatozoa in vitro and antispermatozoic activity in rats in vivo by Terminalia chebula, a flavonoid rich plant. *Reprod Toxicol.* 2010;29:214-24
- [34] Ramaswamy S and Weinbauer G F.(2014). Endocrine control of spermatogenesis: Role of FSH and LH/ testosterone. *Spermatogenesis.* 2014;4:e996025.
- [35] Casarini, L.; Santi, D.; Brigante, G.; Simoni, M.(2018). Two Hormones for One Receptor: Evolution, Biochemistry, Actions, and Pathophysiology of LH and hCG. *Endocr. Rev.* 2018, 39, 549–592.
- [36] Benghuzzi H, Tucci M, Mohamed A, et al.(2018). Differential Histopathological Assessment of Testicular Function Upon Long-Term Exposure to Sustained Delivery of Testosterone and Dihydrotestosterone. *Biomed Sci Instrum.* 2018;54:138-44.
- [37] Olivares V., Barrajon E., Herranz M., et al.(2018). Plant-Derived Polyphenols in Human Health: Biological Activity, Metabolites and Putative Molecular Targets. *Curr Drug Metab.* 2018;19:351-69.
- [38] Sherif sharawy^{1, 2} & Nasir, A. Ibrahim^{1,3} (2014). The Effects of Hibiscus rosasinensis Flower Extracts on Spermatogenesis and Sperm Parameters of Mice. *Global Journal of Biology, Agriculture & Health Sciences. G.J.B.A.H.S., Vol.3(2):32-35 (April –June, 2014) ISSN: 2319 – 5584*
- [39] Prakash Chandra Gupta 2012. Contraceptive potential of Hibiscus rosa-sinensis (Linn.)- An update. *International Journal of Pharmaceutical Research*, 2012, Volume 4, Issue 4, ISSN 0975-2366
- [40] Omer H. Arabi¹, Mutman A. Kahil², Nasir A. Ibrahim³ . The effects of aqueous extract hibiscus sabdariffa on spermatogenesis and sperm parameters of mice. *Int J Cur Res Rev*, May 2014/ Vol 06 (10)
- [41] Nidhi Mishra, Vijay Lakshmi Tandon, Ashok Munjal. Evaluation of Medicinal Properties of Hibiscus rosasinensis in Male Swiss Albino Mice. *International Journal of Pharmaceutical and Clinical Research*; 2009. 1(3): 106-111
- [42] Khulkute, Udupa. Effect of Hibiscus rosasinensis on spermatogenesis in rats. *Planta Medica.* 1977; 31: 127-135.
- [43] Puranik N V, Srivastava P, Bhatt G, et al. Determination and analysis of agonist and antagonist potential of naturally occurring flavonoids for estrogen receptor (ER α) by various parameters and molecular modelling approach. *Sci Rep.* 2019;9:7450.
- [44] Olesen I A, Joensen U N, Petersen J H, et al. Decrease in semen quality and Leydig cell function in infertile men: a longitudinal study. *Hum Reprod.* 2018;33:1963-74.
- [45] Savchuk I, Soder O, Svechnikov K. Mouse leydig cells with different androgen production potential are resistant to estrogenic stimuli but responsive to bisphenol a which attenuates testosterone metabolism. *PLoS One.* 2013;8:e71722.