DOI Number: 10.37506/v20/i1/2020/mlu/194731

Testosterone and Progesterone Levels, Gene Expression of Androgen and Progesterone Receptors in Albino Male Rats Treated with Phenolic Flower Extract of *Hibiscus Rosa - Sinensis* L

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Abstract

Hibiscus *rosa sinensis L.* is one of medicinal plant and belongs to the family Malvaceae , *Hibiscus* species are used in the treatment of many disease, flowers are used to stimulate hair growth , wounds healing activity, anti-parasitic effect , Antidiabetic anti-hyperlipidemic activity. and have many pharmacological properties including anti-fertility , antipyretic, antispasmodic, antifungal, anti-inflammatory and many more. This study was conducted in the department of Biology /College of Science/University of Babylon/Iraq , to evaluate the Effect of Phenolic Extract of *Hibiscus rosa - sinensis* L. flowers on some biomarkers and gene expression in Albino male Rats **.**The overall number of animals used were 32 male rats aged 2-3 months. The results showed a significant decrease ($p<0.05$) in the level of Testosterone and Progesterone in treated rats as compared with control groups for 30 and 60 days. Also the result revealed a significant decrease ($p< 0.05$) in the expression of the androgen receptors and progesterone receptor in the testicular cells treatment group with phenolic compounds of *H. rosa- sinensis* flowers at a dose of 300mg / kg /day of body weight for 60 days compared to control groups.

Keywords: *Phenolic Extract, Hibiscus rosa - sinensis L. Testosterone, Progesterone, gene expression, androgen receptor, progesterone receptor*

Introduction

Many plant extracts can be used as contraceptives by inhibiting the fertility of males and females [1] however, those plant extracts, that have been developed into contraceptive are very few and have not been identified the methods of operation and effectiveness accurately because of the difficulty in finding out the active ingredients of the extract to be used as a herbal contraceptive[2] . Fertility in males is directly affected by the sex hormones produced in the testes, which are called androgens, testosterone is the main androgen of the males, which also produced by the cortex of the adrenal gland. Testosterone begins during embryonic development and last for a short period after birth and stops during the childhood and then produced very significantly at puberty. It is responsible for the growth and development of the male reproductive system, bones, muscles, enlarged throat, change of sound, hair growth and increased sexual desire [3] . Most plants used in the regulation of fertility weaken the process of steroids synthesis by targeting the enzymes involved in this process at the level of leading cells or at the level of hypothalamus- pituitary gland- gonads, it has been shown that many botanical products targeting the Leydig cells and hindering their action [4].The medicinal plant extracts used in Chinese traditional medicine which containing alkaloids as one of the active compounds, caused a decrease in the level of gonadotropin hormones and testosterone which are caused edema in the interstitial tissue of mouse testis treated with these extracts [5]. Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as transfer RNA or small nuclear RNA genes, the product is a functional RNA[6]. In genetics, gene expression is the most fundamental level at which the genotype gives rise to the phenotype. The genetic code stored in DNA is “interpreted” by

gene expression, and the properties of the expression give rise to the organism's phenotype , such phenotypes are often expressed by the synthesis of proteins that control the organism's shape, or that act as enzymes catalyzing specific metabolic pathways characterizing the organism. Regulation of gene expression is thus critical to an organism's development [7].

Materials and Method

The flowers of *Hibiscus rosa –sinensis L.* were collected from nursery and agricultural areas in Babil province in April and May 2018 . The plant was identified by Dr. Neddaa Adnan, plant herbarium/ Department of Biology/ College of Science/ University of Babylon. The flowers were collected in flowering stage after they were well cleaned and washed with water and left to dry in the air at room temperature for two weeks. After that the dried flowers were blended with a blender, until use.

Preparation of crud phenolic compounds of *Hibiscus rosa sinensis L.* flowers

The Crude phenolic compounds were extracted according to [8] method putting 10 gm of powder flower in a flask (500 ml) and added 400 ml of acetic acid 2%. The phenolic compounds of the plant were extracted by using reflex condenser in water bath(70°c) for 8 hours. The solution was leaved to cool down after the completion of extraction. Filtrated by a separatory funnel, and added to it an equal volume of n- propanol and amount of sodium chloride until saturation. Two layers formed, the upper layer containing the phenolic compounds and neglected the lower layer, the upper part has been concentrated in a rotary evaporator then dried in oven (40°c), kept in refrigerator until use .

General phenolic Reagent

This reagent was prepared by mixing two equal quantities of aqueous solutions of 1% ferric chloride and 1% of potassiumic ferric cyanide. The extraction was derided after it was obtained by dry calcium chloride (CaCl2) to get rid of any effect of the moisture which could affect the identification, then the compounds of *H. rosa sinensis* extract was identified by infrared spectrum by using Ft. Infrared spectroscopy [3] .

First experiment : Effect of *H. rosa- sinensis* flowers extract on hormones levels

This experiment include 32 male albino rats divided into two groups

1. Control (n = 16) treated orally with one ml of tap water .

2. Treated group (n = 16) treated orally with 300mg / kg /day of phenolic compounds of *Hibiscus rosa- sinensis* flowers extract .

After 30 days of treatment isolated 8 animals from each group (control and treatment) blood sample had been taken from animals for evaluated: Testosterone and progesterone hormones instruction of kits(Maglumi Testosterone CLIA/ China) .The other animals (included 8 control and 8 treatment) , was continue treatment for 30 other days , so that the treatment become 60 days . same biomarkers estimation were done like the first period (30 days) .

Second experiment : Genetic study

Control group : (n = 3) treated orally with 1 ml of tap water for 60 days

Treated group: (n = 3) treated orally with 300 mg/ kg /day phenolic compounds of *Hibiscus rosa- sinensis* flowers for 60 days .

Tissue preparation

Testis of each rat were collected immediately. Half of the sample had been frozen and stored at –80C until analysed.

Total RNA extraction and Real-Time PCR.

Total RNA was extracted from the samples according to the protocol of TRIzol™ Reagent (Thermo Scientific, USA).Complementary DNA (cDNA) was constructed by reverse transcription (Promega Corp., Madison, WI, USA) using the total RNA as a template .The primers used for rat AR,PR and glyceraldehyde 3-phosphate dehydrogenase (G3PDH) as a reference gene in RT-PCR were as follows : AR forward,5-TGTTATCTAGTCTCAACGAGC-3; AR revers,5-CATCATTTCAGGAAAGTCC-3; PR forward, 5-CTCCTGGATGAGCCTGA TG-3; PR reverse,5-CCCGAATATAGCTTGACCTC-3,G3PDH forward,5-TCCCATCACCATCTTCCA-3 and G3PDH reverse,5-CATCACGCCACAGTTTCC-3.The PCR system consisted of 5 µl of SYBR Green qPCR mix; 0.25 µl of cDNA,0.25 Mgcl2,2.5 Nuclease Free Water and primer pairs (0.5 µM forward and 0.5 µM revers) in a total volume of 10 µl. PCR was performed using standard protocols using the annealing temperature for 30 s at

52 c° .A final extension cycle was performed at 72 c° for 30 s .The melting curve program was 72-95 c°, with a heating rate of 0.3°C/s and continuous fluorescence measurement , the expression level of these genes was evaluated by an image software .

Results and Discussion

The effect of phenolic extract of *H .rosa sinensis L.* on Testosterone , Progesterone hormones level :-

Table 1 showed that the treatment of Albino rats with a dose of 300 mg/kg/day of body weight of phenolic compounds of *H. rosa- sinensis* flowers caused a significant decrease ($p<0.05$) in the level of Testosterone and Progesterone in treated rats compared with control groups. The level of this hormones in both groups in comparssion study between 30 days and 60 days of treatment revealed insignificant differences ($P > 0.05$) between two period for each hormones (Figure1,2). These results are consistent with the results of [9 , 10] , which revealed that the phenolic compounds of *H. rosa- sinensis* caused decreased level of testosterone in serum of treated rats. The reason for the low testosterone level may due to the effect of *H. rosa- sinensis* extract reducing the level of cholesterol and low density lipoproteins (LDL) in the blood [11] . As the cholesterol derived from LDL is the basic compound in the process of testosterone synthesis in Leydig cells [12] The phenolic compounds of green tea reduced the level of testosterone through their effect on the enzymes required to the synthesis of hormone such as P450scc and 53βHSD [13]. Also the low level of testosterone may be due to the effect of phenolic compounds of *H. rosa sinensis* flowers on Leydig cells , some plant extracts have a negative effect on the numbers and diameters of Leydig cells and their nucleus, causing a reduction in the production of testosterone [14,15].

The low level of testosterone may be due to the effect of extract on the metabolic pathways of testosterone resulting from the presence of secondary compounds, especially flavonoids found in the *H.rosa sinensis* , Ohno *et al* [5] pointed out that the Genistein compound, a type of flavonoids found in ginseng plant leads to the reduction of testosterone in patients of prostate cancer, this effect has been attributed to the chemical composition of this compound which is similar to the steroid hormones such as estrogen, and thus it somehow affects the process of hormonal regulation in the body and leads to lower levels of testosterone [16]. The exposure to high concentrations of external estrogen hormone can cause changes in the levels of gonadotropin hormones and reproductive function [17].The ingestion of phytoestrogen by experimental animals caused a significant reduction in LH and FSH secretion and decrease the level of testosterone [18]. The Progesterone enhances sperm count , libido and increasing muscle mass , it also increase testosterone levels in the body and enhance its effects , being that it's a precursor of the mineralocorticoid aldosterone, and androstenedione which can be converted to testosterone , estrone and estradiol [19] . In current study showed significant decrease in the level of progesterone in treated animals with phenolic extract of *H . rosa* .The treatment of male rats orally at a dose of 500 mg/kg of body weight of aqueous extract of *H. rosa sinensis* flowers caused a significant decrease in the concentration of sperms in testes and epididymis [20]. Progesterone in male produce from adrenal gland, adrenal steroid biosynthesis is regulated by a negative feedback system, in which corticotrophin-releasing hormone secreted from the hypothalamus activates adrenocorticotropic hormone release from the pituitary, and ACTH then stimulates the zona fasciculate cells to produce cortisol [21]. The reduction in Progesterone and Testosterone level in serum may be due to the inhibitory effect of the phenolic compounds of *H. rosa sinensis* flowers of the hypothalamic- pituitary- testicular axis, since some studies have indicated the effect of different plants extracts on inhibiting the secretion of hormones such as alcoholic extract of *Mentha arvensis* leaves , which caused a significant reduction in the level of LH in the serum of treated male rats due to the inhibitory effect of the extract of the hypothalamic – pituitary – testicular axis [22].

Table 1. The effect of *H .rosa sinensis L.* flowers extract on male rats for 30 and 60 days.

Parameters	30 days		60 days	
	Control (mean ± SE)	**Treated (mean ± SE)**	**Control (mean ± SE)**	**Treated (mean ± SE)**
Testosterone (ng /ml)	a 3.22 ± 0.12	b 2.02 ± 0.27	a 3.06 ± 0.13	b 1.66 ± 0.12
Progesterone (ng/ml)	a 1.53 ± 0.11	b 0.97 ± 0.22	a 1.35 ± 0.12	b ± 0.14

-Different letters mean a significant difference (p > 0.05) between groups .

The effect of phenolic extract of *H.rosa sinensis* on androgenn receptors AR and progesterone receptor PR.

The results of this study showed a significant decrease (p< 0.05) in the expression of the androgen receptors (AR) and progesterone receptor (PR) of the testicular cells in group of rats treated with phenolic compounds of *H. rosa- sinensis* flowers (300mg / kg /day of body weight) for 60 days compared to control groups(Figure 3,5) .The AR is a ligand- dependent transcription factor that is activated when androgens were found , anti- androgens or AR antagonists cause prevention of androgens for carrying out their biological activity via directly binding and blocking the AR receptor ligand binding domain (LBD), and by inducing repressive activity [23] . The mechanism of anti- androgens action begin when anti- androgens bind to the binding pocket of the AR and thereby its activation [24].

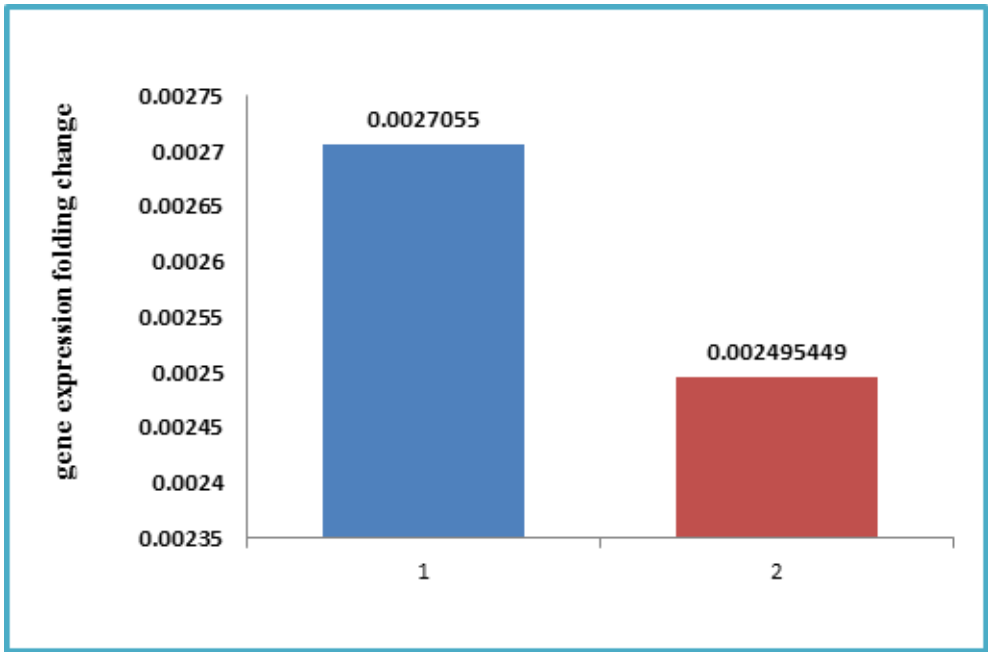

Figure 1: Folding change in gene expression of androgen receptor gene in treated and control rats groups during 60 days .

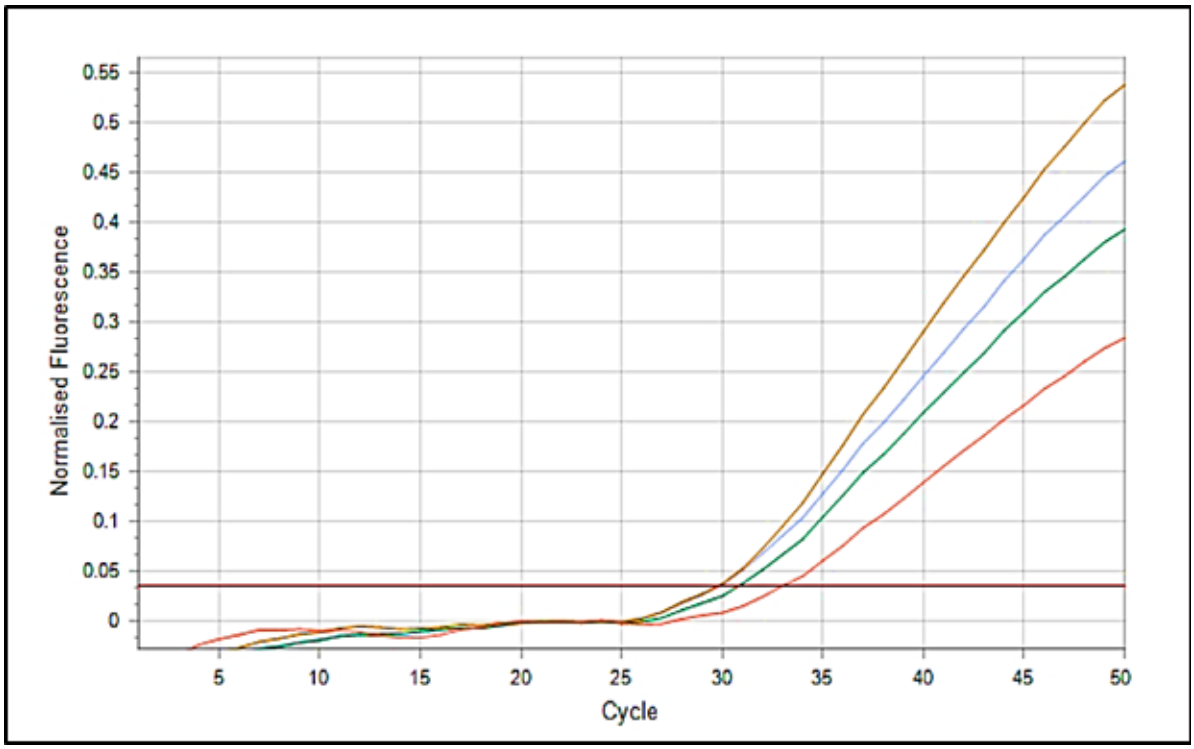

Figure 2: Real-Time PCR amplification plot of androgen receptor in testis

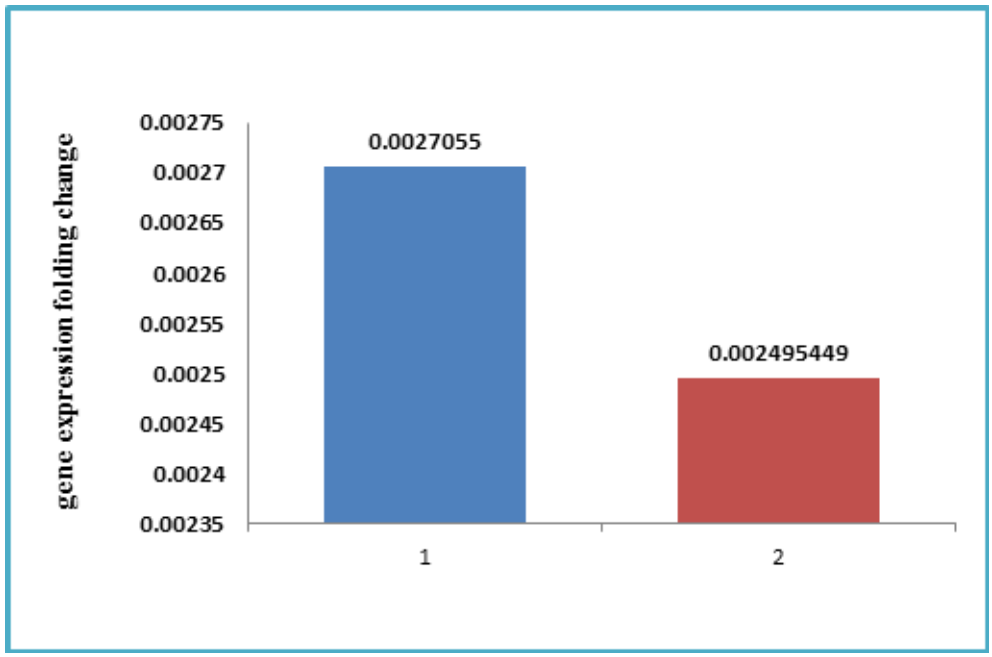

Figure 3: Folding change in gene expression progesterone receptor gene treated and control rats groups during 60 days .

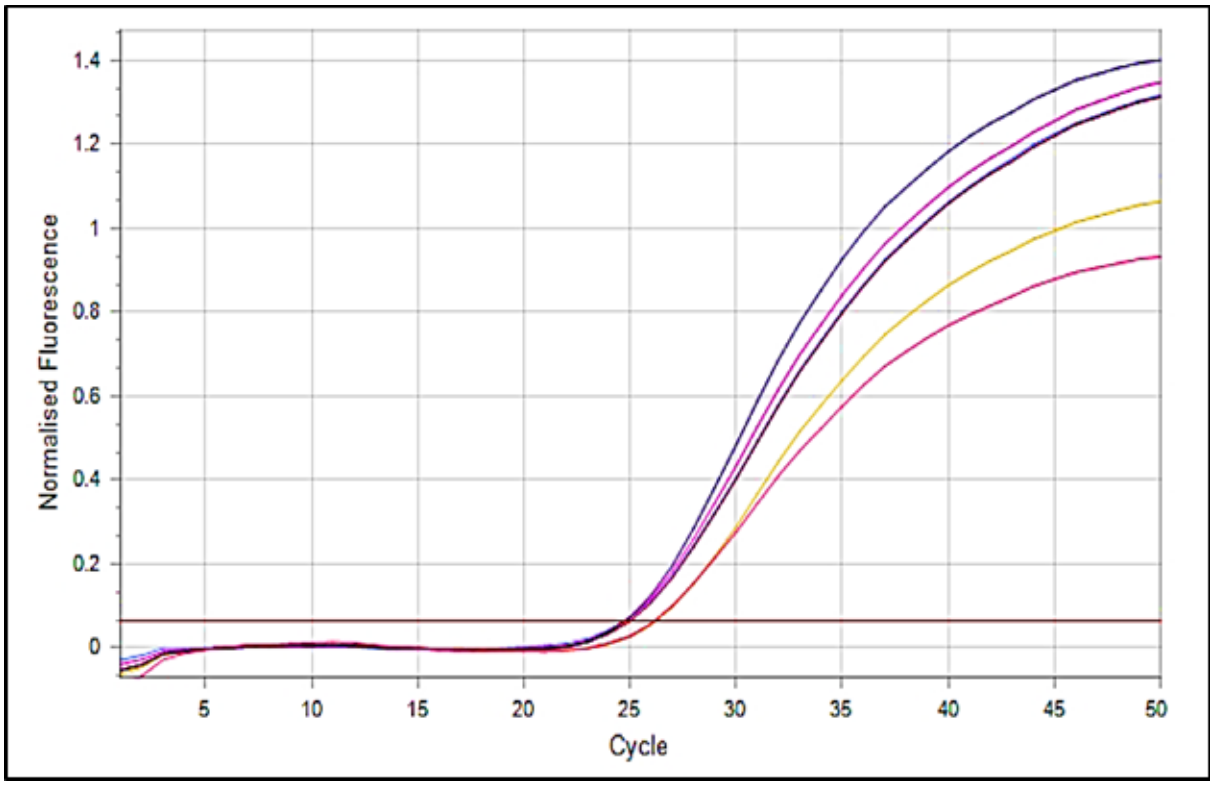

Figure 4: Real-Time PCR amplification plot of Progesterone receptor gene in testis .

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Veterinary Medicine, AL-Qasim Green university- Babylon-Iraq and all experiments were carried out in accordance with approved guidelines.

References

1. Remya M , Sharma RC , Deepali M ,Sakshi B, Nilesh P,Tharini S. In vitro effects of Aegle marmelos on human sperm vitality . Biomedicine., 2009;29(2):183-85.
2. Ghosh D , Jana D , Debnath JM. Effeats of leaf extract of Stephania hernandifolia on testicular gametogenesis and androgenesis in albino rats: a dose -dependent response study. Contraception, 2002;65(5):379-84.
3. Ganong WF. Review of medical physiology. 23rd Ed. Lange medical Books/ McGraw Hill. New York, Chicago, San Francisco, Lisbon London, Madrid, Mexico city, Milan, New Delhi, San Juan, Seoul, Singapore, Sydney, Toronto. 2010
4. Bhatt N , Chawla SL , Rao MV. Contraceptive evaluation of seed extracts of Abrus precatorius (L.) in male mice (Mus musculus). Herb Med Toxicol., 2007;1: 47-50.
5. Ohno S , Nikalima Y , Inoue K , Nakazawa H , Nakajin S. Genistein administration decrease serum corticostreone and testosterone levels in rats. Life. Sci., 2003;74(6):733-42.
6. Brueckner F. Structure–function studies of the RNA polymerase II elongation complex. Acta Crystallographica. 2009;65 (2): 112–120.
7. Jump up S, Busse D ,Dittmar G , Schuchhardt J , Wolf J. Corrigendum: Global quantification of mammalian gene expression control. Nature.2013;495(7439):126–137.
8. Shriner R, Hermann C. The systematic identification of organic compounds. 8th ed., John Wiley & Sons. Inc. 2004.
9. Al-Saily HM..Effect of Hibiscus rosa sinensis L. extract and cyproteron acetate on fertility , some immune functions and cell culture in male albino rats . Athesis of ph.d , College of science- university of Babylon. 2018.
10. Al- Zubaide BA. Effect of the phenolic extract of Hibiscus rosa sinensis L. flowers in some

histilogical and physiological of reproduction male albino rats. Athesis of ph.d , College of science-university of Babylon .(in arabic). 2014.

11. Sachdewa A , Khemani LD. Effect of Hibiscus rosasinensis Linn. Ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. J Ethnopharmacol., 2003;89:61-6.
12. Beamer WG , Wilson MC , Leiter EH. Endocrinology in: The mouse in Biomedical Research. (Foster, H.L.; Small, J.D. and Fox, J.G.). American College of Laboratory. New York. 1983; 218 -224.
13. Figueiroa MS , Juliany SB, Vieira C, Leite DS, Ruben CO. Green tea polyphenols inhibit testosterone production in rat Leydig cells Asian J. of Andrology, 2009; 11: 362–370.
14. Parandin R , Sadeghipour HR , Haeri-Rohani SA. Evaluation of antifertility Meffect and recovery of the seed oil constituents of Iranian species of Melia azadrach L. in male rats" J. of Reproduction & Contraception, 2008; 19(3):161-166.
15. Mathur N, Jain GC, Pandey G. Effect of Tecoma stans Leaves on the Reproductive System of Male Albino Rats .International J. of Pharmacology, 2010;6: 152-156.
16. Kumar NB , Canto A , Allen K.The specific role of isoflavones in reducing prostate cancer risk. Prostate, 2004;59: 141-147.
17. Kilian E ,Delport R , Bornman MS , Jager C. Simultaneous exposure to low concentrations of dichlorodiphenyl- trichloroethane, deltamethrin, nonylphenol and phytoestrogens has negative effects on the reproductive parameters in male SpraqueDawley rats.Andrologia, 2007;39: 128–135.
18. Tai M, Zhang J, Song S. Protective effects of luteolin against acetaminophen-induced acute liver failure in mouse. Int Immunopharmacol. 2015 ; 271:164–70.
19. John RL. Testosterone, male menopause and hormone balance in men. New Yorker magazine. 2012.
20. Mishra RK , Singh SK. Safety assessment of Syzygium aromaticum flower bud (clove) extract with respect to testicular function in mice. Food Chem Toxicol., 2008;46(10):3333-8.
21. Veldhuis JD ,et al. Age-dependent and gender-dependent regulation of hypothalamic-adrenocorticotropic-adrenal axis. Endocrinology and metabolism clinics of North America. 2013;42 (2): 201-25.
22. Sharma N , Jacob D.Anti-fertility investigation and toxicological screening of the Petroleum ether extract of the leaves of *Mentha arvensis* L. in male albinomice. J. Ethnopharmacol., 2001*;75*: 5-72.
23. Halsen C , Kerkhofs S , Clinckemalie L , Spans L. Strctural basis for nuclear hormone rerceptor DNA binding. Mol. Cell. Endocrinol., 2012;348(2):411-417.
24. Lans C. Ethnomedicines used in Trinidad and Tobago for reproductive problems. J. of Ethnobiology and Ethnomedicine, 2007 ;(3):113.