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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 OlmiumSandum, Piperlongus, Cotton seed oil extract and Ayurvedic ingredients possess antifertility activity [6, 7]. Antifertility effect of *HibisacusSabdariffa* 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. *HibisacusSabdariffa* 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], *Lepidiummeyenii*lipidic extract [11], *Turneradiffuse* and *Pfaffiapaniculata* extracts [12], *Tribulusterrestris* extract [13], *Panaxginseng* roots [14] *Eurycomalongifolia* extract [15] *Terminalacatappa* seeds extract [16], *Rutachalepensis*agueous extract [17], Polysaccharides of *Lyciumbarbarum* fruit extract [18], *Shengjingpill*; a Chinese formula of plant extracts [19], *Hibiscusmacranthus* and *Basellaalba* aqueous extracts [20], *Crotonzambesicus*ethanolic extract [21], and *Astragalusmembranaceus* and *Acanthopanacissenticosi*aqueous extracts [22]. However, the antifertility activity is 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 competive binding of hormones on immbilised 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

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expressed as mean ± 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 thensections 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 th**e** 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 ± SEM (n=10)

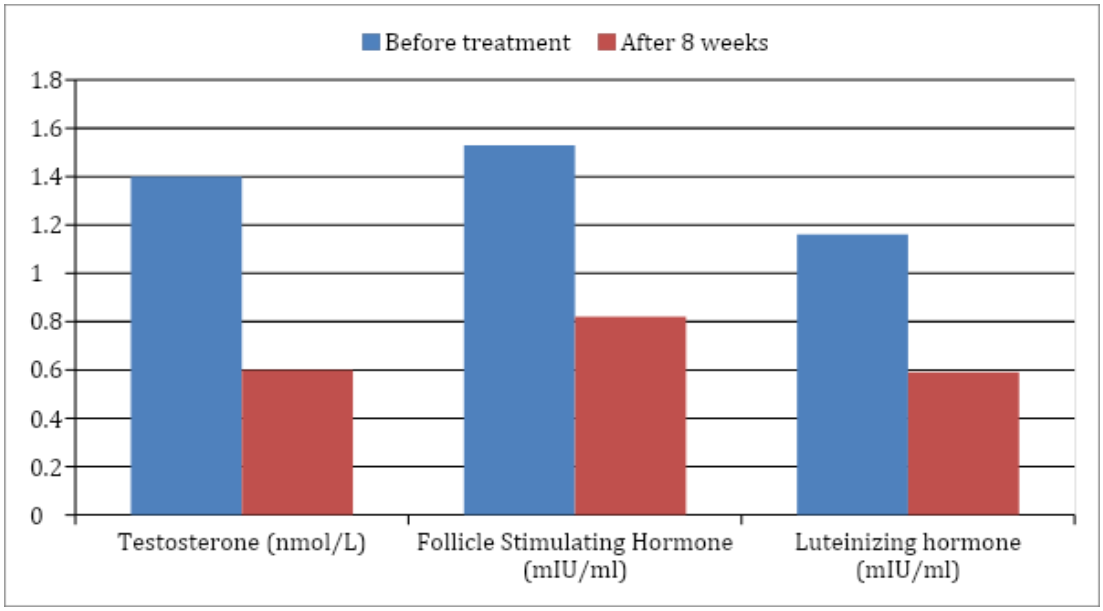

Fig1. Effect of *Hibiscus sabdariffa* calyx extract on circulating levels of selected male rabbits reproductive hormones

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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)

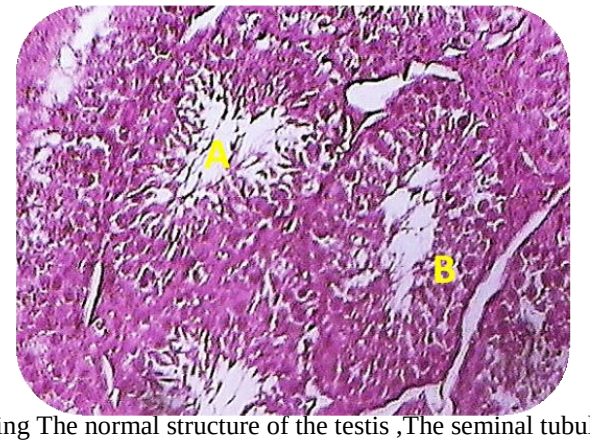

Fig.1 section of testes showing The normal structure of the testis ,The seminal tubules appear of regular shapes and sizes (A), as well as being lined with several rows of spermatogenic cells (B)

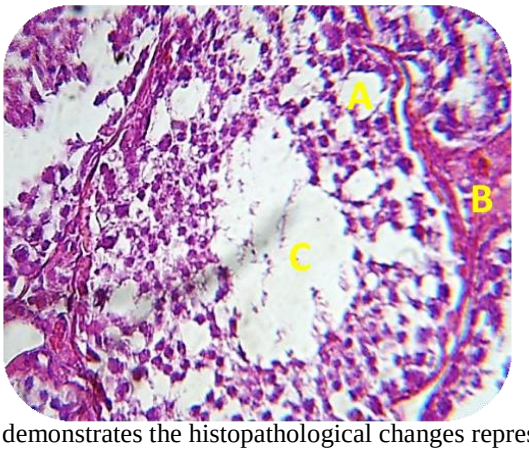

Fig.2 Section of testes showing demonstrates the histopathological changes represented by detachment of the connecting tissue between cells (A), congestion of some blood vessels between the tubules (B), irregularity of the lumen of the seminal tube and the absence of sperms (C).

Discussion

Adrenal cortex, gonads, and placenta are considered endocrine glands that secret different steroidal hormones that produce their effect on target organs [23]. Testosterone is produced by the leydig cells, and has hormonal and prohormonal characteristics, and 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

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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: tannius, alkabidstriterpenoids, flavonoids, taraxeyl acetates Poly phenols, saponinshibisetin, cyaindine glycosides, ca-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 rosasinunsis 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 pituary adrenal and pituary 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 occure 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 transfort 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.

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