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A new approach of herbal contraception in male Swiss albino mice by *Hibiscus rosasinensis*: An in vivo study

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ABSTRACT

The experiment was conducted to explore the contraceptive effect of the *Hibiscus rosasinensis* on the testes of the male Swiss albino mice. Twenty (20) Swiss albino mice (male) of 30 days of age (avg. b.wt. 25-28 gm) were purchased from ICDDR,B, Mohakhali, Dhaka, Bangladesh and they were divided into two groups as: Control group (C) and the treated group (H) each group having 10 mice (6 male and 4 female). The control group was fed with the normal feed (mice pellet) and water *ad libitum;* and the treated group was fed with the *Hibiscus rosasinensis* extract @ 500 gm/mice in 20 ml of distilled water by medical micro dropper. The experiment was started at the age of 60 days (as at age of 45-48 they reach to the puberty, before that the mice were reared up to 48 days to observe the normal fertility of the male and female mice and accustomed with the environment as well). The experimental tenure was 42 (uniformity of management for all the groups were maintained) days as three (3) successive cycles were observed and after the treatment the mice were sacrificed ethically and the sample (testes) was collected for the anatomical (gross and histological) observation. The histological observation of the testes revealed that in the treated group (H) the number of the seminiferous tubules reduced and the amount of spermatozoa within the lumen of the seminiferous tubules also decreased. The number of the sertoli and leydig cells also reduced within and between the seminiferous tubules along with slight deposition of fat droplets and vacuolation. The ultimate result of the research divulged that the extract of the *Hibiscus rosasinensis* had a potential impact on the testis regarding the contraceptive effect as in the treated group the female did not conceive after keeping with the treated male.

INTRODUCTION

Nature is the source of all the raw materials that are needed for the human being (Anil and Ashatha, 2012). About 2-3 decades ago, most of the drugs were of herbal origin. A variety of reasons remain behind why people like to use the natural medicines as it is the evidence that the patients are getting even more distressed after using the chemically synthesized drugs, rather the natural means such as seed, fruit, cleaves and plants that can conquer detrimental diseases as well as the controlling birth, leaving no baleful effects on the human health. For birth control or contraception there are various methods such as: vasectomy in males and tubal ligation in females, use of intrauterine devices (IUDs), contraceptive pills/birth control pills and implantable contraceptives. Birth control pills are the chemical or synthesized form of contraceptive which prevent the pregnancy secondarily by changing the uterine lining and cervical mucus to make it harder for sperm to reach the uterus and for an embryo to implant. Several dietary and herbal supplements can interfere with the efficacy of birth control pills (Michele Noonan, 2013*)*.Unfortunately there is no effective herbal contraceptive material for controlling the birth in male, although in some previous studies, it was revealed that by using different herbal products we can control the birth in both male and female and some plants, seeds and flowers can also be used for this purpose (Kadiri et al., 2009). There are also many herbs that are very innocuous, which can be used for birth control by virtually anyone without any negative effects (Hannah ransom, 2013 and Okoko et al. 2010). But, still there is no such potential research on the use of *Hibiscus rosasinensis* in contraception.

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Hibiscus rosasinensis (Family: Malvaceae) is a perennial ornamental plant available throughout the Indian subcontinent.

Flower of *Hibiscus rosasinensis*

The active constituents of the plant include β sitosterol, stigmasterol, taraxeryl acetate and three cyclopropane compounds and their derivatives in leaves and stems. Flowers contain cyanidin diglucoside, flavonoids and vitamins, thiamine, riboflavin, niacin and ascorbic acid (Ghani, 2003). Quercetin-3-diglucoside, 3,7-diglucoside, cyanidin-3,5-diglucoside and cyanidin-3-sophoroside-5-glucoside have been isolated from deep yellow flowers; all above compounds and kaempferol-3-xylosylglucoside have been isolated from ovary white flowers (Rastogi & Mehrotra, 1993).

Various parts of this plant, like leaves, flowers and roots, have been known to possess medicinal properties like aphrodisiac, menorrhagia, oral contraceptive, laxative, etc. It is a medicinal plant having many effects among them antifertility is a remarkable one. However, *Hibiscus rosasinensis* has this antifertility or contraception effect that has been used from the ancient time, but exact histological features to find out the efficacy of the plant on the male reproductive organ (testes) has not been found out yet. The present study was aimed to determine the contraceptive effect of *Hibiscus rosasinensis* on testes of male Swiss albino mice.

MATERIALS AND METHODS

Experimental animal

Swiss albino mice model was used for this experiment which were purchased from the ICDDR,B, Dhaka, Bangladesh. The onset of the experiment was at 60 days as both the male and female mice reach to the sexual maturity or puberty at the age of 45-48 days. The average weight of each mouse was 25-28 gm. Total 20 mice were used in the experiment among them 12 were male and 8 were female and they were divided into 2 groups each group having 10 mice (6 male & 4 female). Both the control and treated groups mice were reared in the Lab animal house, Dept. of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh and they were supplied with normal mice pellet and water *ad-libitum*. The room was well ventilated and temperature was maintained at 25-30^0 C (Fathia et al., 2005). The uniformity of all the management practices was also maintained.

Plant extract

The plant that was used for the purpose was *Hibiscus rosasinensis* L. (Joba). The plant material (flower) was collected from the local garden and air dried for 10 days under an open shade and pulverized with the help of a mortar and pestle to fine powder. Fifty 500 mg of powder was dissolved in 20 ml of distilled water in a conical flask. The mixture was intermittently shaken throughout the period of extraction using glass rod stirrer but allowed to stand overnight and filtered with whatman filter paper No 1 into measuring cylinder and concentrated at 60^{0}C in an incubator and next stored in a refrigerator at 4°C until required for use (Ajagbonna et al., 2002).

Experimental procedure

Before the onset of the experiment the mice were accustomed with the laboratory condition for 15 days by providing standard mice pellet and water *ad libitum*. During this period the regular record of the feed consumption and body weight gain was kept as the evidence of proper health. At the 60th day age of the mice the treatment with the aqueous extract of the *Hibiscus rosasinensis* was started.

The aqueous extract of *Hibiscus rosasinensis* was given @ 1ml/mice (500 mg powder/kg b.wt.) and administration was done orally with the help of medical micro dropper in both male and female of the treated group (H). The mice of the treated group (H) were fasted over night before the day of administration of the aqueous extract. After 7 days of first dose the mice were given the second dose (both the cases the dose was similar). Normal feed (mice pellet) and *ad libitum* water were provided in control group. During this period the normal feeding and body weight gain measurement were continued.

Collection of the sample

The histology of the testes was done and for the purpose after the ethical sacrificing of the mice (male) of both the treated and control groups, the testes was collected, processed and stained with routine haematoxylin and eosin stain (H & E) for microscopic observation.

RESULTS AND DISCUSSION

In the histological section (stained with haematoxylin and eosin) of the testis (Figure 1) showed a deep red stained outer fibrous layer, the tunica albuginea, beneath which the seminiferous tubules cut in various plains were found in the control group (C). Each seminiferous tubule was lined by several layers of cells (spermatogenic), which when viewed under high power magnification reveals an indistinct of cellular boundary. The outer rows of nuclei belong to the sustentacular cells and spermatogonia. From inward towards the center of the tubules, large nuclei of spermatocytes and many smaller nuclei of spermatids were found. Also, huge numbers of the sertoli and leydig cells were present within and between the seminiferous tubules.

In case of the treated group (H) testis, variations in the histological features were found from that of the control. The number of the seminiferous tubules reduced in a single focus. In the control group (C), the number of the seminiferous tubules were 35 in a single focus whereas, in the treated group (H) the number reduced and it became 28 in a single focus (Figure 2)

The amount of the spermatozoa within the lumen of the seminiferous tubules also decreased. In the control, the amount was about 75% whereas in the treated group (H) the amount decreased to 65% in a single focus within the lumen of the seminiferous tubules (Figure 2).

The amount of the sertoli cells within the seminiferous tubules and leydig cells in between the seminiferous tubules also decreased in the treated group (H) comparing to the control testis of the group C (Figure 3)

The aforesaid results reflect the effects of the *H. rosasinensis* on the testes as recognized by a decreased sperm production indicating a lower fertility tendency in the male.

Moreover, in an observation Figure 2 the seminiferous tubules decreased in the thickness. Also, a lower density of the germinal epithelium and hypertrophy in majority of the cells were observed. Finally, the lumen shows negligible presence of sperms in the treated animal as compared to the control.

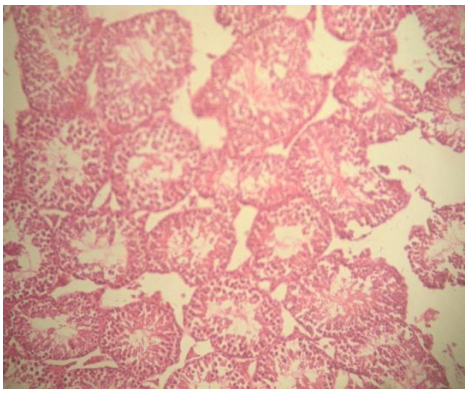

Figure 1
Histological architecture of the testis of the control group (C) showing the normal features of the testis (H & E, X 10)

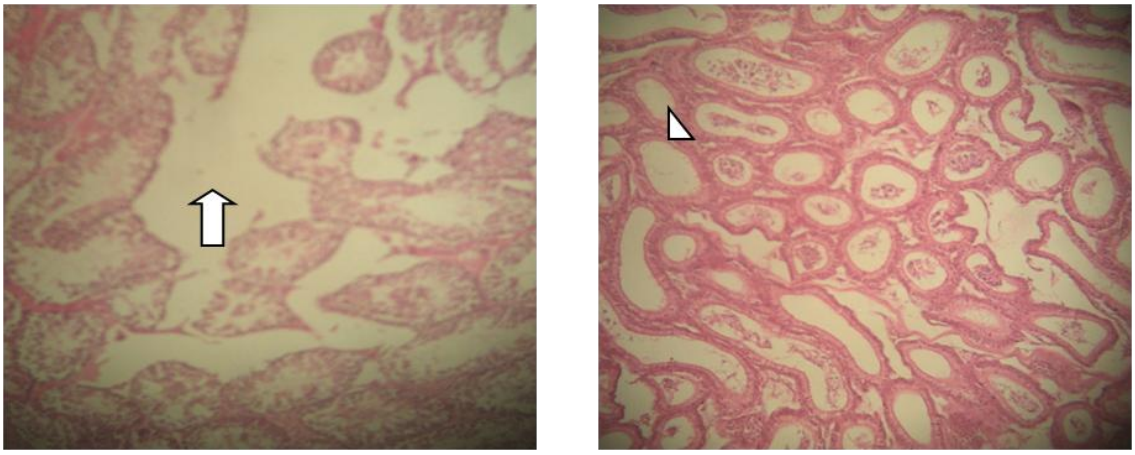

Figure 2
Histological architecture of the testis of the treated group (H) showing the reduced number of seminiferous tubules (arrow) and decreased number of the spermatozoa within the lumen of the seminiferous tubules (arrow head) (H & E, X 10).

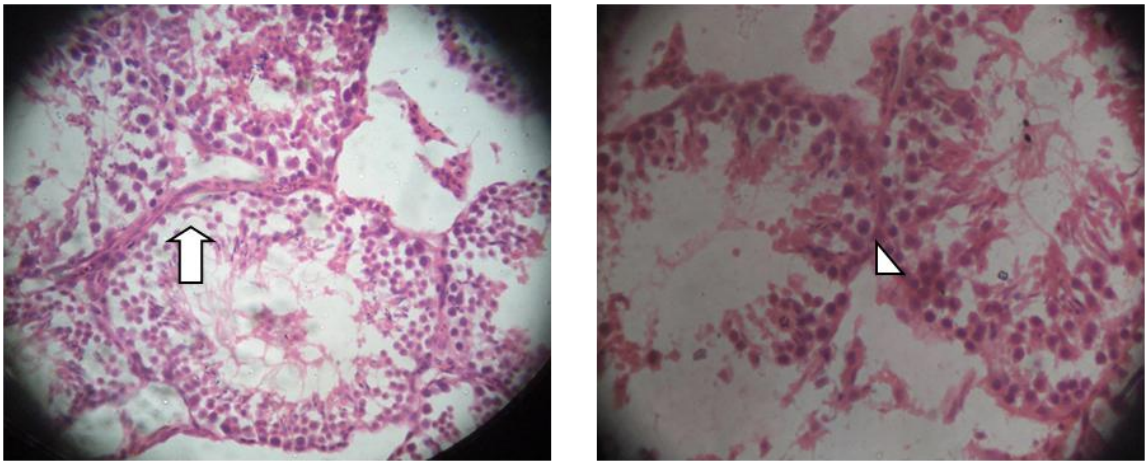

Figure 3
Histological architecture of the testis of the treated group (H) showing the decreased number of sertoli cells within the seminiferous tubules (arrow) and decreased number of the leydig cells in between the seminiferous tubules (arrow head) (H & E, X 40).

The histological result of this study revealed that the number of the seminiferous tubules reduced and the thickness also reduced in the treated group (H) comparing to the controls (C), that was similar to that of the findings of Sherif and Nasir (2014), who reported that histological testis in mice treated with the plant extract of *Hibiscus rosasinensis* showed alterations in the seminiferous tubules including decreased in thickness and density of germinal epithelium and hypertrophy in majority of spermatogenic cells in the treated male mice as compared to control.

The amount of the spermatozoa in the lumen of the seminiferous tubules also decreased, which was supported by Ekbujo et al., (2008) as the scientists of that research found the same but they did their research on wistar mice. The number of the sertoli and leydig cells within and between the seminiferous tubules reduced which was resembled as the findings of Sherif and Nasir (2014). The 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 albino rats (Ekbujo et al., 2008).

Possible pathways of causing contraception in mice

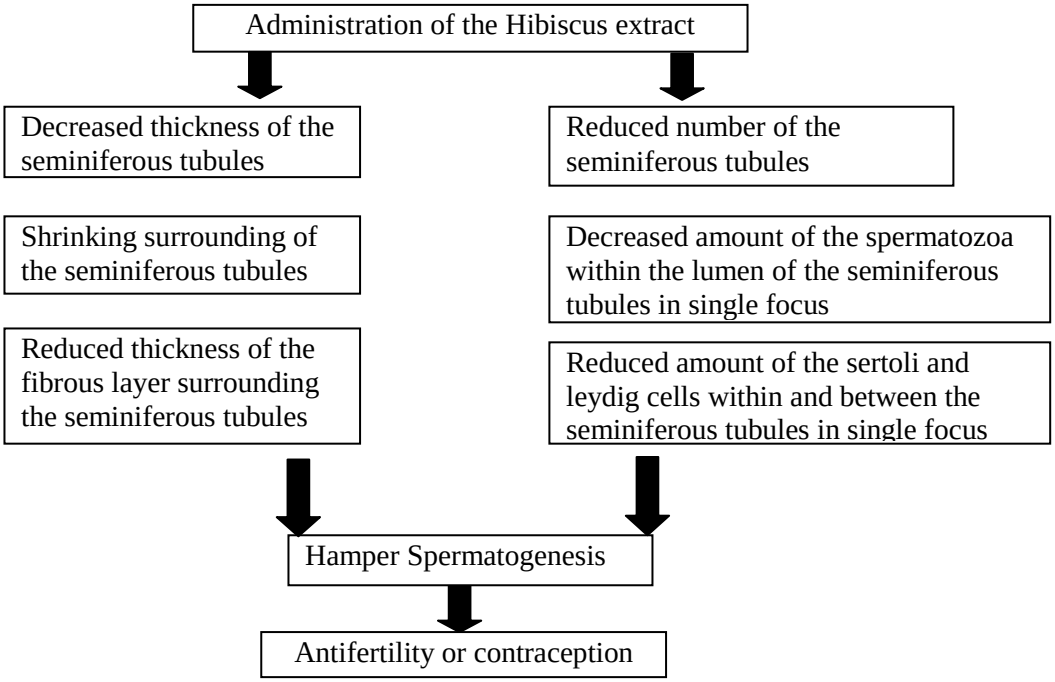

Figure 4: Effect of *Hibiscus rosasinensis* extract in contraception

CONCLUSION

Considering the aforesaid results it might be praiseworthy to mention here that *Hibiscus rosasinensis* seems to hold great potential for in-depth investigation for contraception. The authors hope to attract the attention of the natural product researchers throughout the world to focus on this unexplored potential of *Hibiscus rosasinensis*, and it may be useful in developing new formulations with more therapeutic values for exploring new, cheaper, available and above all safe herbal contraceptives especially in male.

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