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The Effects of *Hibiscus rosa sinunsis* Flower Extracts on Spermatogenesis and Sperm Parameters of Mice

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Abstract

This study was aim to study the effect of ethanol, chloroform, ethyl acetate extract of *Hibiscus rosa sinunsis* on spermatogenesis and sperm parameters on mice. Adult mice (n=20) were included in the present study. Thereafter, the mice were randomly divided into control (n=5) and experimental groups (n=15). Treated with dose 125mg/Kg of ethanol, chloroform and ethyl acetate extract (injected subcutaneous) for three consequence days. The study was showed adverse effect of extracts of *Hibiscus rosa sinunsis* on sperm parameters and histology of testis.

Keyword: *Hibiscus rosa sinunsis*, ethanol, chloroform, ethyl acetate extract, spermatogenesis.

Introduction

Hibiscus rosa-sinunsis (Fam. Malvaceae) is a perennial plant available throughout world. Various parts of this plant, like leaves, flowers and roots, have been known to possess medicinal properties like aphrodisiac, menorrhagia, oral contraceptive, laxative, etc.(Ladd J.L. , *et al* 1978). Several articles and ancient literature have shown that the flowers of this plant possess antifertility activity, like antimplantation, abortifacient, in rodents (Murthy D.R.K. 1996). Implantation is a very crucial event in reproductive physiology. Several biochemicals, biophysical and hormonal changes take place prior to this event.(Richard J.S. ,1980) Several studies have shown that endometrial membrane conditions are important for blastocyst implantation progesterone, estrogen, oxyradical and antioxidant systems regulate implantation. The aqueous-ethanolic extract of aerial parts of *H. rosa sinensis* was reported for its use in constipation and diarrhea (Anil K and Ashatha S ,2012). *Hibiscus rosa sinesis* is a native of china and is a potent medicinal plant. It is a common Indian garden perennial shrub (Mudgal. (1974). And often planted as a hedge or fence plant. *Hibiscus rosa sinensis* flower decoctions are used in India and Vanuatu as aphrodisiacs, for menorrhagia, uterine haemorrhage and for fertility control (Lans, 2006). Hibiscus rosa-sinensis had also been used as fertility control agent in some animal models (Zhou (1998), Jiang (1998), Tan (1983), Farnsworth (1982) and Tiwari (1982). The flowers have been reported to posses anti-implantation and anti-spermatogenic activities (Murthy *et al* 1997). Vasudeva and Sharma (2007) reported a post-coital activity of ether extract of *Hibiscus rosa-sinunsis* roots administered orally to colony-breed female albino rats(Wister strain) and adult albino mice.

Materials and Methods

Extract preparation

The plant extracts from *Hibiscus rosa-sinunsis* flower was prepared using the solvents, ethanol, Acyle acetate and chloroform. 50g of the samples were taken and homogenized with 100ml of the respective solvents. The crude preparation was left overnight in the shaker at room temperature and then centrifuged at 4000rpm for 20 mins. The supernatant containing the plant extract was then transferred to a beaker and the extract was concentrated by evaporating the solvent at 60°C. The crude extract was weighed and dissolved in a known volume of distal water to obtain a final concentration of 125mg / kg

Experimental animals

Adult mice (n=20) were included in the present study. The mice were 8 weeks old with average weight 28±3g each. Male mice were housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/ dark cycle prior to experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. All mice were fed a standard diet. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Thereafter, the mice were randomly divided into control (n =5) and experimental groups (n =15). However, the experimental groups split into two groups each included five mice. Treated with dose 125mg/Kg of ethanol, chloroform and ethyl acetate extract (injected subcutaneous) for three consequence days.

Epididymis sperm count, viability and motility

Sperms from the cauda epididymis were released by cutting into 2 ml of medium containing 0.5% bovine serum albumin .After 5 min incubation at 37°C (with 5% CO2), the cauda epididymis sperm reserves were determined using the standard hemocytometric method and sperm motility was analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO method .

Histopathology and Light microscopy

The testis were fixed in 10% formalin and embedded in paraffin. Five-micron thick sections were prepared and stained with Hematoxylin and Eosin (H&E). The specimens were examined under Olympus/3H light microscope-Japan.

Statistical analysis

Statistical comparisons were made using the ANOVA test for comparison of data in the control group and the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). Significant difference is written in parentheses.

Result

Spermatogenesis and Sperm Parameters:

The result obtained was found average of body weight increased in ethanol and chloroform extract groups and there was no significant difference at level ($p>0.05$) and it showed significant decreased at level ($p<0.05$) in ethyl acetate extract when compared with the control group (Table .1). The results showed that there was high significant difference at level ($p<0.05$) testis weight in chloroform extract and ethyl acetate extract when it showed there was no significant difference at level ($p>0.05$) in ethanol extract compared with the control group. Sperm counts was significant difference at level ($p<0.05$) in ethanol and chloroform extract groups and high significant difference at level ($p<0.05$) in ethyl acetate extract. Sperm motility was high significant difference at level ($p<0.05$) in chloroform extract and ethyl acetate extract and significant difference at level ($p>0.05$) in ethanol extract compared with the control group. Sperm viability was high significant difference at level ($p<0.05$) in ethanol and chloroform extract and significant difference at level ($p>0.05$) in ethyl acetate extract compared with the control group.

Histology investigations

Histology of male mice treated with Hibiscus rosa sinunsis daily for three days control transverse section of control testes showing normal seminiferous tubules collagen tissue spermatogonia cell (Fig.1.) When transverse section of ethanol extract testes showing seminiferous tubules reminded intact and distortion in arrangement of cell spermatogonia series and distortion in seminiferous tubules (Fig.2.) and transverse section of chloroform extract testes showing seminiferous tubules with widened lumen distortion in seminiferous tubules and distortion in arrangement of cell spermatogonia series (Fig.3.) then transverse section of ethyl acetate extract testes showing normal seminiferous tubules collagen tissue and intact and distortion in arrangement of cell (Fig.4.).

Discussion

Historically, the medicinal value of plants was tested by trial and error, as in the Doctrine of Signatures. Modern approaches to determining the medicinal properties of plants involve collaborative efforts that can include ethno botanists, anthropologists, pharmaceutical chemists, and physicians. Tan. CH, (1983) and Nidhi *et al*. (2009), report that, *Hibiscus rosa sinensis* extracts had no effects on weights of the male reproductive organ in a study of rats this result similar the result in present study. Sperm parameters result obtained in the present study were consistent with the finding by Farnsworth *et al.* (1982), Temitope (2010). Who found that alcoholic extracts of *H. sinensis* flowers led to decreased spermatogenic elements of testis and epididymal sperm count. The histological result in this study was approved by Nidhi M *et al* (2009), who report that histological, testis in mice treated with the plant extract showed alteration in the seminiferous tubules and alteration include decrease in thickness and density of germinal epithelium and hypertrophy in majority of cells moreover lumen shows negligible presence of sperms in the treated animal as compared to control. Crude extract of blooms of *Hibiscus rosa sinensis* has been demonstrated that there was definite antifertility effect of this extract in causing degenerative changes in the germinal epithelium of male albino rats (Kholkute, 1977).

Conclusion

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 rosa sinunsis* on sperm parameter and histology of testis.

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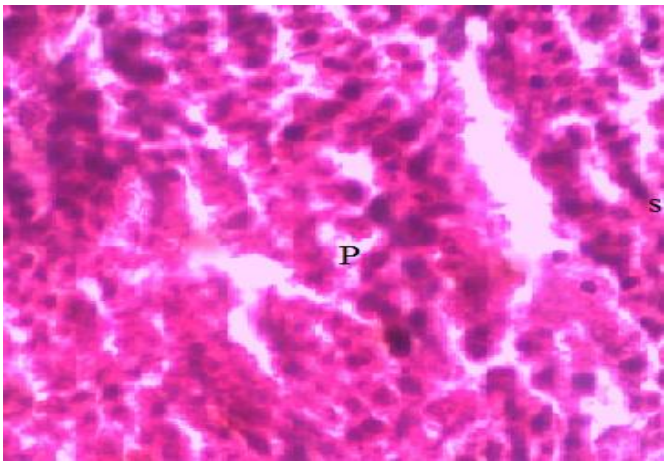
Table.1. Effects of *Hibiscus rosa sinunsis* on the average of body, testis weight (g), sperm count (10^6/ml), sperm motility (%) and sperm viability (%)

Parameter	Control	Ethanol extract	Chloroform extract	Ethyl acetate extract
Body (gr)	22.8000± 0.86	23.78±0.37	21.80±1.49	18.80±1.62*
Testis (gr)	0.45±0.02	0.44±0.20	0.54±0.17**	0.52±0.14**
Sperm concentration (total count) (No of sperm/rat 10^6)	53.60±4.40	48.20±3.58*	45.60±7.25*	37.00±2.88**
Motility (%)	39.00±1.76	34.60±3.60*	47.00±9.92**	30.40±1.80**
Viability(%)	55.20±4.31	39.20±4.87**	34.40±2.46**	45.80±5.31*

*= significant; ** High significant

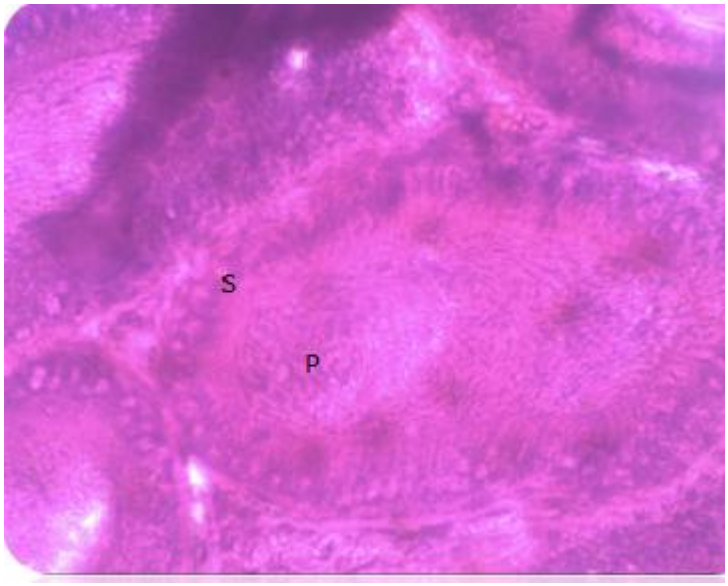
S= seminiferous tubules

Fig.1. Control section of control testes showing normal seminiferous tubules collagen tissue spermatogonia cell.



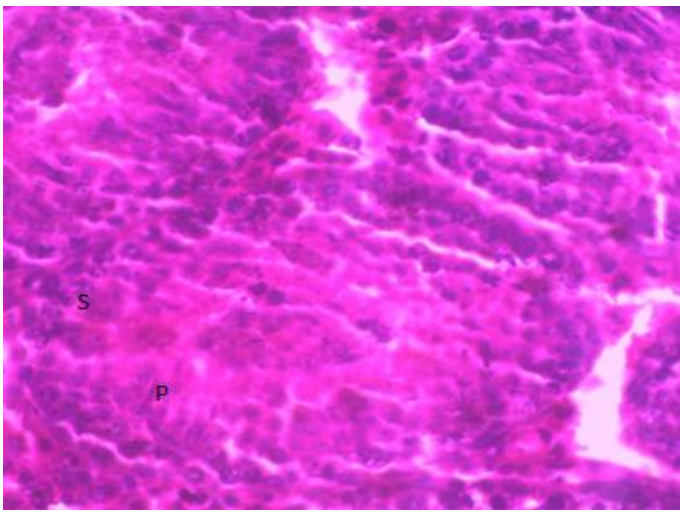
S= seminiferous tubules; P= spermatogonia

Fig.2. section of ethanol extract testes showing seminiferous tubules reminded intact and distortion in arrangement of cell spermatogonia series and distortion in seminiferous tubules.



S= seminiferous tubules; P= spermatogonia

Fig.3. section of chloroform extract testes showing seminiferous tubules with widened lumen distortion in seminiferous tubules and distortion in arrangement of cell spermatogonia series.



S= seminiferous tubules; P= spermatogonia

Fig.4. section of ethyl acetate extract testes showing normal seminiferous tubules collagen tissue and intact and distortion in arrangement of cell.