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THE EFFECTS OF AQUEOUS EXTRACT *HIBISCUS SABDARIFFA* ON SPERMATOGENESIS AND SPERM PARAMETERS OF MICE

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ABSTRACT

This study was aim to evaluate the effect of aqueous fruit extract of *Hibiscus sabdariffa* Linn on spermatogenesis and sperm of mice. Adult male mice (n=30) included in the present study. The mice were eight weeks old and their average weight was 28±3g. Male mice housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/ dark cycle according experimental protocols. Thereafter, the mice were randomly divided into control (n=10) and experimental group (n=20). The control group received daily 8 ml distilled water, group-1 of the experimental group received 50 gm/Kg/BW and group-2 received 100 gm/Kg/BW of aqueous extract of *Hibiscus sabdariffa*. The experiment extended for 21 days. The results of this study showed that there was an increase in the average body weight in the experimental groups compared with the control group with a significant difference (p<0.05). While the testis weight, sperm counts, sperm motility and viability decreased in the experimental groups compared with the control group with a significant difference (p<0.05). The effect of *Hibiscus sabdariffa* on Sex hormones the study showed that there was significant different (p> 0.05) in FSH; LH and testosterone hormones when compared with control In study of testis histology showed a significant decrease in the lumina spermatozoa. The study concluded that *Hibiscus sabdariffa* has adverse effect on spermatogenesis and sperm parameters of mice.

Keywords: Aqueous, *Hibiscus sabdariffa*, spermatogenesis, sperm parameters, mice

INTRODUCTION

The roselle (*Hibiscus sabdariffa*) is a species of Hibiscus native to the Old World tropics, used for production of bast fiber and as an infusion (Chau, J. W *et. al.* 2000). The calyces, which are rich in phenolic compounds contain gossypetin, glucoside, hibiscin, hibiscus anthocyanin and hibiscus protocatechuic acid, diuretic and choleric effects, decreasing viscosity of the blood, reducing blood pressure and stimulating intestinal peristalsis (Ali and Salih, 1991; Owulade

et al., 2004). Tseng *et al.*, (1996) found that the mechanism of this protective effect associated with the scavenging of free radicals Hibiscus protocatechuic acid also inhibits lipopolysaccharide induced rat hepatic damage. Although the mechanism of action of *H. Sabdariffa* as a cholesterol-lowering agent was not elucidated in this work, it hypothesized, albeit with no experimental evidence, that the extract may contain some compounds that activate hormonal secretions, such as adrenocortical

hormones, which stimulate the metabolic pathway of cholesterol by conversion into other compounds. The anti cholesterol action of *Hibiscus sabdariffa* (0.5% or 1%) confirmed in rabbits fed cholesterol for 10 weeks. This treatment was effective in reducing the serum concentrations of triglycerides, total cholesterol and low-density lipoprotein cholesterol, and in mitigating atherosclerosis in the aorta (Chen *et al.*, 2003). Kanokwan Sukjail and Ampa Luangpirom (2010) studied aqueous seed extract of *Hibiscus sabdariffa* in male rats, and found that all treated groups showed decreasing of sperm concentration, percentage of normal motile sperms and vital sperms, while the percentage of abnormal sperms increased. The lactogenic effect of ethanol seed extract of *Hibiscus sabdariffa* was also found in both sexes of albino rats, and median lethal dose (LD₅₀) of this extract was above 5000 mg/kg BW of rats (Gaya I, *et al.* 2008). In Nigeria, *Hibiscus sabdariffa* Linn. Seed tea was used traditionally to enhance lactation in case of poor milk production in human (Gaya I, *et al.* 2008).

MATERIALS AND METHODS

Experimental animals

Adult mice (n=30) included in the present study. The mice were 8 weeks old with average weight 28±3g each. Male mice housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/ dark cycle according to experimental protocols. All animals treated in accordance to the Principles of Laboratory Animal Care. All mice fed a standard diet. The daily intake of animal water monitored at least one week before start of treatments to determine the amount of water needed per experimental animal. Thereafter, the mice were randomly divided into control (n=10) and experimental groups (n=20). The control group received daily 8 ml distilled water. However, the experimental groups split into two groups each included ten mice. Group-1, received 50 gm/Kg/BW and group-2, received 100

gm/Kg/BW of *Hibiscus sabdariffa* for 21 consecutive days.

Epididymis sperm count, viability and motility

Sperms from the cauda epididymis 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 determined using the standard hemocytometric method and sperm motility analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO method.

Hormones measured

Serum collected at termination used for assaying for total testosterone, FSH and LH. Testosterone, FSH and LH measured using a commercial ELISA kit. Which is based on competitive binding of hormones on immobilised antibody. Horse radish peroxidase was used for color development and absorbance at 420nm measured on a plate reader. Values are reported as ng/ml of serum.

Histology and Light microscopy

The testes fixed in 10% formalin and embedded in paraffin. Five-micron thick sections prepared and stained with Hematoxylin and Eosin (H&E). The specimens 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 expressed as mean ± S.E.M (standard error of means). Significant difference is written in parentheses.

RESULT

Spermatogenesis and Sperm Parameters:

Effects of *Hibiscus sabdariffa* on Spermatogenesis and Sperm Parameters summarized in table.1.&2 the obtained data shows that there were statistically high significant (p<0.05) decrease in body, testis weight, sperm counts, sperm motility and viability in all experimental groups, including

50gm/Kg/BW and 100gm/Kg/BW the roselle - treated compared with the control group. In sex hormones the study showed that there was significant different ($p < 0.05$) in FSH, LH and testosterone hormones when compared with control.

Histology investigations

Histological observations regular seminiferous tubule with normal germinal epithelium morphology in control sections (Figure.1.). Treated mice showed regular seminiferous tubule with normal germinal epithelium morphology. Sperm presence in lumen and was significantly decrease in the lumina spermatozoa at 50 mg/kg/BW and 100 mg/kg/BW concentration of *Hibiscus sabdariffa* (Figure. 2. A and B).

DISCUSSION

Herbal medicine is based on the premise that plants contain natural substances that can promote health and reduce illness (Craig, 1999). Titilayo O.*et al.*, (2008) who report that the rat received 2000 mg/kg dose of 50% ethanol extracts experienced consistent loss of weight. There were statistically high significant decrease in body, testis weight, sperm counts, sperm motility and viability in present study. This result was consistence by Kanokwan Sukjail and Ampa Luangpirom (2010) who studied the effect of aqueous seed extract of *Hibiscus sabdariffa* Linn, in male rates and found that impairment of spermatogenesis by decreasing of sperm concentration, percentage of normal motile sperms and vital sperms, while the percentage of abnormal sperms increased. Also, finding by Yomna, I (2010) whom were found that the effect of different doses of *H. sabdariffa* Linn. either cold or boiled, alter normal sperm morphology and testicular ultrastructure and adversely influence the male reproductive fertility in albino mice. In the present study sex hormones FSH, LH and testosterone was significant different. This result was not consistenc with result obtained by Omotuyi, *et al* (2010) whom reported that Serum

levels of follicle stimulating hormone (FSH) in male rabbits administered 200 mg/kg body weight oral doses of anthocyanin preparations with time. In water control group, FSH rose steadily from day 0 to day 7, remained fairly constant till day 21 and decreased rapidly to the lowest on day 28. Groups administered with anthocyanin-extract showed gradual rise from day zero to day 28. Serum FSH of anthocyanin control increased gradually from day 0 to day 21 and decreased slightly on day 28 and Serum levels of testosterone in male rabbits administered 200 mg/kg body weight oral doses of anthocyanin preparations with time. The water-control group showed fairly constant testosterone levels throughout the experimental period. For anthocyanin control group, testosterone levels decreased from day 0 to day 21 and increased to the peak on day 28. Anthocyanin extract showed decreased serum testosterone from day 0 to day 7 and increased steadily to the peak value on day 28. This trend is similar to that of group administered whole-extract. In histological investigation the lumen of seminiferous tubules showed a significant decrease in the lumina spermatozoa. This result was not similar with the result obtained by Orish E, *et al* (2004) who found that in the study testicular effects of sub-chronic effect of *Hibiscus sabdariffa* (HS) calyx aqueous extract on the rat testes. 1.15 g/kg dose group showed distortion of tubules and a disruption of normal epithelial organization, while the 2.3 g/kg dose showed hyperplasia of testis with thickening of the basement membrane. The 4.6 g/kg dose group, on the other hand, showed disintegration of sperm cells. The effects observed with most of the plant and plant-based products have been attributed to the antispermatogenic and/or antisteroidogenic properties of one or more active ingredients. (Shereen C, *et al*, 2010)

CONCLUSION

The results of this study showed that there is an increase in the average body weight in the

experimental groups with a significant difference ($p < 0.05$). While the testis weight, sperm counts, sperm motility and viability decreased in the experimental groups with a significant difference ($p < 0.05$). Sex hormones FSH, LH and testosterone was significant different ($p < 0.05$) In histological study showed that the lumen of the seminiferous tubules was a significant decrease in the luminal spermatozoa. The study concluded that *Hibiscus sabdariffa* has adverse effect on spermatogenesis and sperm parameters of mice.

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Table.1. The effects of Aqueous extract *Hibiscus sabdariffa* on sperm parameters of mice

Parameter	Control(n=10)	G. 1 <i>Hibiscus sabdariffa</i> (50mg/kg-/day) (n=10)	G.2 <i>Hibiscus sabdariffa</i> (100mg/kg/day) (n=10)
Body (gr)	28.20±0.67	31.00±0.56*	31.8±0.39*
Testis (gr)	0.20±0.081	0.14±0.056*	0.16±0.064*
Sperm concentration (total count) (No of sperm/rat 10 ⁶)	65.30±3.06	32.9±0.99*	19.87±1.78*
Motility (%)	35.79±2.36	27.1±0.35*	8.60±0.62*
Viability(%)	65.26±3.06	28.87±0.73*	11.19±1.16*

Significant* (P<0.05)

Table.2. The effects of Aqueous extract of *Hibiscus sabdariffa* on Sex hormones

Parameter	Control(n=10)	Experiment Group
FSH (ng/ml)	0.11±0.07	0.19±0.01*
LH(ng/ml)	0.14±0.02	0.15±0.03*
Testosterone (ng/ml)	0.33±0.09	0.17±0.02*

Significant * (P<0.05)



Figure 1: Regular seminiferous tubule with normal germinal epithelium morphology, in control group. (X400).

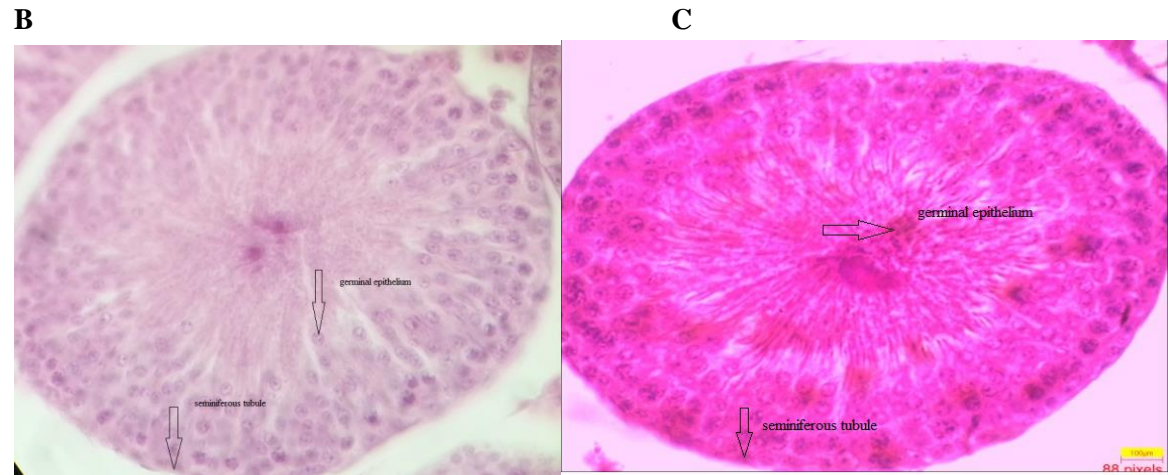


Figure 2. B & C. Regular seminiferous tubule with normal germinal epithelium morphology and sperm presence in lumen and a significant decrease in the luminal spermatozoa in 50gm/Kg/BW and 100gm/Kg/BW roselle. (X400).