

Biochemical Effect of *Hibiscus sabdariffa* Calyx Extracts on the Reproductive Hormones of Male Wistar Rat

Chukwunonso K. Nwabuo* and O Olusanya

Department of Biochemistry, Faculty of Life sciences, University of Benin, Nigeria

ABSTRACT

Hibiscus sabdariffa Linn. (Roselle) is an annual shrub which is popular in Asian and African countries including Nigeria. It is used as a medicinal plant for the treatment of various disease conditions such as hypertension and hyperlipidemia.. This study was undertaken to investigate the biochemical effect of *Hibiscus sabdariffa* calyx extract on male rat reproductive hormones. Twenty (20) male Wistar rats weighing between 100 g-200 g were grouped into a control group and three experimental groups. While the water control group received 1.0 ml of water for 28 days, the experimental groups were administered 250 mg/kg oral doses of aqueous extract of *H. sabdariffa* calyx. The effect of the extract on the basal levels of follicle stimulating hormone, Testosterone, Prolactin, Luteinizing hormone and Estradiol were conducted in experimental animals. The 28 days administration of aqueous extract of *H. sabdariffa* L. is associated with decreased circulating plasma levels of follicle stimulating hormone, Testosterone, Luteinizing hormone and Prolactin in male wistar rat compared with the control group. The study did not show a significant change in the plasma levels of Estradiol following 28 days oral administration of *H. sabdariffa* calyx to the experimental animal. The study concluded that *H. sabdariffa* calyx extract at a dose of 250 mg/kg caused significantly reduced the levels of Follicle stimulating hormone, Testosterone, luteinizing hormone and prolactin in male Wistar rat, mild effects on rat reproductive hormones.

Keywords: *Hibiscus sabdariffa*; Follicle stimulating hormone; Prolactin; Testosterone; Luteinizing Hormone; Estradiol

INTRODUCTION

Hibiscus sabdariffa Linn. (Roselle) is an annual shrub which is popular in Asian and African countries including Nigeria. The brilliant red colour of its calyx makes it a valuable food product, apart from its multitude of traditional medicinal uses [1]. Infusions of the calyces are considered as diuretic, cholerectic, febrifugal and hypotensive, decreasing the viscosity of the blood and stimulating intestinal peristalsis [2]. The medicinal properties of *Hibiscus sabdariffa* Calyx (HSc) have been associated with certain phytochemical constituents. Some of the phytochemical constituents responsible for the pharmacological effect of *Hibiscus sabdariffa* include flavonoids and protocatechuic acid. Most of these phytochemical constituents act by disrupting the endocrine functions. Extracts of *H. sabdariffa* calyces have been reported to be rich in phytoestrogens [3-6] which have endocrine disrupting effects.

In Nigeria, a drink commonly called “Zobo” (a sweetened aqueous extract of *Hibiscus sabdariffa*) is gradually gaining ground in the market place as a more preferred substitute for carbonated drinks and for medicinal purposes without consideration of the body’s physiological state [7] Previous studies have shown that HSc caused significant reduction in levels of circulating reproductive hormones(1, 6). However, little or no information have been provided about the testicular of aqueous extract of HSc.

This study was undertaken to determine the extent to which aqueous extract of HSC alters the basal levels of selected reproductive hormones: Follicle stimulating hormone (FSH), Prolactin (PRL), Testosterone (TST), Luteinizing hormone (LH) and Estradiol (E2) in male wistar rats.

MATERIALS AND METHOD

Preparation of aqueous extract of *Hibiscus sabdariffa* calyx

Dried calyces of *Hibiscus sabdariffa*, was obtained from a local market in Benin City, Edo state, Nigeria and authenticated at Plant Biology and Biotechnology Department, University of Benin, Benin city, Edo state, Nigeria. Two hundred grams (200g) of dried HSc was boiled in 500 ml of distilled water for 15 min. The boiled sample was allowed to cool and then filtered into a sterile bottle and refrigerated.

Experimental animals

A total of 20 male wistar rats weighing 100 g-200 g were housed in a clean animal house and fed rat chow and water *ad libitum*. Rats were acclimatized for a period of 14 days under standard environmental conditions.

Experimental design

Male wistar rats were divided into four groups each of five. Group one is the water control and the other three groups received 250 mg/kg of plant extract through a gavage daily for 28 days.

Collection of blood sample

Blood samples were collected from conjunctival veins using capillary tubes at the end of the 28 days of oral administration of *H. sabdariffa* calyx extract.

Hormonal assay

The hormones were estimated using the standard protocols of Enzyme-linked immunosorbent assay (ELISA) Kits for the determination of FSH, PRL, TST, LH and E2 follicle stimulating hormone (FSH), Prolactin (PRL), Testosterone (TST), Luteinizing Hormone.

Statistical analysis

All the obtained data were expressed as means \pm standard deviation and analyzed using Analysis of Variance (ANOVA). Comparison with the control groups was made using One-way ANOVA. Differences were considered significant if P-value $<$ 0.05.

RESULTS

The oral administration of 250 mg/kg of aqueous *H. sabdariffa* calyx extract for 28 days resulted in a significant decrease ($P < 0.05$) in the circulating levels of FSH, PRL, TST, LH and E2.

However, 250 mg/kg of the extract did not have a significant change ($P > 0.05$) in the circulating levels of Estradiol (Table 1).

Table 1: Effect of *Hibiscus sabdariffa* calyx extract on circulating levels of selected rat reproductive hormone (U/L)

S/N	Groups	FSH	PRL	TST	LH	E2
1	Control	45 \pm 0.70711	26 \pm 1.41421	1.5 \pm 0.15811	30 \pm 2.70185	5 \pm 1.00000
2	250 mg <i>H. sabdariffa</i> /kg b.wt	36 \pm 0.70711	21 \pm 1.64317	1.0 \pm 0.14142	24 \pm 1.70294	3 \pm 0.92087

Values are expressed as mean \pm SEM (n=5)

DISCUSSION

Phytoestrogens such as isoflavones and lignins have been investigated for their estrogenic properties [8-10]. There are several reports showing that HSc is rich in phytoestrogen [3-6]. There are reports indicating that HSc calyx have estrogenic effects, although the exact phytochemical constituent responsible for the estrogenic property has not been determined. There are studies showing that plant phenols, anthocyanin isolates and anthocyanin-rich mixture of bioflavonoids also possess estrogenic activities [6].

In this present study, 28 days oral administration of 250 mg HSc extract resulted in a decrease in plasma levels of circulating FSH in male rat compared with the control group. This observation was reported by Omotuyi et al. and Arabi et al. [6,11]. This result was not consistent with the report by Sirag et al. [1].

To explain this observation, decrease in circulating FSH would be traced to the imbalance of synthesis and clearance of follicle stimulating hormone [6].

The study showed a decrease in circulating levels of plasma TST in male rat following 28 days oral administration of 250 mg of HSc extract. This observation was reported by Sirag et al. [1], Arabi et al. [6] and Omotuyi et al. [11].

This observation may be explained by the estrogenic activity of the plant. This evidence was raised by Orisakwe et al. [4]. Furthermore, other studies had implicated phytoestrogens as being responsible for the decrease in TST levels in laboratory animals treated with phytoestrogens [12,13]. The main role that oestrogens play in male reproductive development is unclear, but oestrogens tend to have 'demasculinising' or antiandrogenic effects [1]. In foetal and neonatal life, this probably results from suppression of TST production or when androgen receptors becomes unavailable at the target site [14,15]. The enzyme Aromatase catalyzes the synthesis of Oestrogens from androgens and there is a close relationship between the mechanisms of action of these two hormones. Furthermore, TST may be converted to estrogen by aromatase [16].

FSH is a known regulator of testicular development and it also increases spermatogenesis and steroidogenesis [6]. It therefore follows that a decrease in FSH triggers a decrease in TST.

The decrease in circulating plasma prolactin following 28 days oral administration of 250 mg HSc extract can be traced to the activity of prolactin-inhibiting hormone, dopamine and clearance of prolactin by specific tissues such as ovary, liver, kidney and mammary gland [17,18]. The level of prolactin in the plasma at any time is therefore influenced by the factors affecting synthesis and clearance.

The decrease in the levels of circulating luteinizing hormone could be attributed to an imbalance in the synthesis and clearance of Luteinizing Hormone.

It should be noted that in carrying out studies involving plant extracts, one cannot attribute the observed biological effects to a particular constituent because many other compounds are present in the plant extracts [19]. Certain Factors such as species, age, gender, diet, dose, route of administration and metabolism generally influence the ultimate biological response to phytoestrogen exposure [20].

CONCLUSION

The study concluded that 28 days oral administration of HSc extract to male Wistar rats had a significant effect on the circulating levels FSH, PRL, TST and LH. Further studies are necessary to understand the molecular mechanism responsible for the alteration in the levels of circulating rat reproductive hormones.

REFERENCES

- [1] Sirag N, Elhadi MA, Algaili MA, Hozeifa M, Imad MT. Effect of *Hibiscus sabdariffa* calyx extract on reproductive hormones in normal rat. *Afr J Pharm Pharmacol*, **2013**, 7: 2295-2298.
- [2] Salleh N, Runnie I, Roach D, Mohamed S, Abeywardena Y. Inhibition of low-density lipoprotein oxidation and up-regulation of low-density lipoprotein receptor in HepG2 cells by tropical plant extracts. *J Agric Food Chem*, **2002**, 50: 3693-3697.
- [3] Adigun MO, Ogundipe OD, Anetor JI, Detunde AO. Dose-dependent changes in some haematological parameters during short-term administration of *Hibiscus sabdariffa* aqueous extract (zobo) in wistar albino rats. *Afr J Med Med Sci*, **2006**, 35: 73-77.
- [4] Orisakwe OE, Husaini DC, Afonne OJ. Testicular effects of sub chronic administration of *Hibiscus sabdariffa* aqueous extract in rats. *J Reprod Toxicol*, **2004**, 18: 295-298.
- [5] Brian J, Jonna F, Lauren E, Alice P, Gail B. Estrogenic effects of herbal medicines from Costa Rica used for the management of menopausal symptoms. *J Menopause*, **2009**, 16: 748-755.
- [6] Omotuyi IO, Ologundudu A, Onwubiko VO, Wogu. *Hibiscus sabdariffa* Linn. anthocyanins alter circulating reproductive hormones in rabbits. *J Diabetes Endocrinol*, **2011**, 1: 36-45.
- [7] Iyare EE, Adegoke OA. Gestational outcome in rats that consumed aqueous extract of *Hibiscus sabdariffa* during pregnancy. *Pakistan Journal of Nutrition*, 10: 330-354.
- [8] Miksicek RJ. Estrogenic flavonoids: Structural requirements for biological activity. *Proc Soc Exp Biol Med*, **1995**, 208: 44-50.
- [9] Collins BM, Mclachlan JA, Arnold SF. The estrogenic and antiestrogenic activities of phytochemicals with the human estrogen receptor expressed in yeast. *Steroids*, **1997**, 62: 365-372.
- [10] So FV, Guthrie N, Chambers AF, Carroll KK. Inhibition of proliferation of estrogen receptor-positive MCF-7 human breast cancer cells by flavonoids in the presence and absence of excess estrogen. *Cancer Lett*, **1997**, 112:127-133.

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- [11] Arabi OH, Mutman AK, Nasir AI. The effect of aqueous extract of *Hibiscus sabdariffa* calyx on spermatogenesis and sperm parameters. *Int J Cur Res Rev*, **2014**, 6: 51-56.
- [12] Sharpe RM, Martin B, Morris K, Walker M. Infant feeding with soy formula: Effects on the testis and blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity. *J Human Reprod*, **2002**, 17: 1692-1703.
- [13] Cline JM, Franke AA, Register TC, Adams MR. Effects of dietary isoflavones aglycones on the reproductive tract of male and female mice. *J Toxicol Pathol*, **2004**, 32: 91-99.
- [14] Haavisto T, Numela K, Pohjanvirta R, Huuskonen J. Prenatal testosterone and LH levels in male rats exposed during pregnancy to 2,3,7,8-tetrachlorodibenzo-p-dioxin and diethylstilbestrol. *J Mol Cell Endocrinol*, **2001**, 178: 169-179.
- [15] McKinnell C, Atanassova N, Williams K, Fisher JS, Walker M, et al. Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol. *J Androl*, **2001**, 22: 323-338.
- [16] Benassayag C, Perrot-Appianat M, Ferre, F. Phytoestrogens as modulators of steroid action in target cells. *J Chromatogr B*, **2002**, 777: 233-248.
- [17] Colao A, Lombardi G. Growth-hormone and prolactin excess. *Lancet*, **1998**, 352: 1455-1461.
- [18] Brook GD, Hindmarsh PC. *Clinical Pediatrics and Endocrinology*. Blackwell Science, Oxford, **2001**.
- [19] Saied-Karbalay D, Ali N, Farzaneh D, Mohammad R. Effects of hydroalcoholic extracts of *Matricaria chamomilla* on serum testosterone and estradiol levels, spermatozoon quality and tail length in rat. *Iran J Med Sci*, **2010**, 35: 25-32.
- [20] Thakur S, Bawara B, Dubey A, Nandini D, Chauhan NS, et al. Effect of *Carum carvi* and *Curcuma longa* on hormonal and reproductive parameter of female rats. *Int J Phytomed*, **2009**, 1: 31-38.