Hibiscus Sabdarrifa L. Anthocyanins-Induced Changes in Reproductive Hormones of Cadmium-Exposed Rats

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Abstract- *Hibiscus sabdarrifa L.* extracts contain powerful antioxidants that can ameliorate Cadmium (Cd)-induced reproductive toxicity, though the active molecules responsible have not been categorically stated. This study is therefore aimed at exploring changes induced by *H. sabdariffa L* anthocyanins (HSA) in reproductive hormones of cadmium-exposed rats. Twenty four adult male wistar rats (185±5.2g) were randomly divided into four groups and where treated for 15 days: A: control, B: Cd alone, 3mg/kg b wt, C: HSA alone, 3mg/kg b wt, Group 4: HSA Pre-CD: HSA (3g/ kg b wt for ten consecutive days) and Cd (3mg/ kg b wt) for five days. Comparison of the serum testosterone level of the control group with that of the Cd alone group showed a significant reduction ($P<0.05$) in the level of serum testosterone but the administration of HSA before Cd exposure significantly reverted the decrease in serum testosterone. Conversely, there was significant increase ($P<0.05$) in the level of serum follicle stimulating hormone (FSH) after cadmium administration (Group C) compared to animals in the control group (Group A) and those maintained on only *H. sabdariffa* anthocyanin alone. This decrease in serum follicle stimulating hormone (FSH) occasioned by Cd exposure was significantly ($P<0.05$) reversed by the administration of *H. sabdariffa* anthocyanin before Cd exposure. This was also observed for Luteinizing hormone. The results confirm the reproductive toxicity of Cd and the reported antioxidant capacities of anthocyanins. In addition, it gives credence to prominent role attributed to HSA in the antioxidant prowess of *H. Sabdarriffa* extracts.

Index Terms- Antioxidants, testosterone, follicle stimulating hormone, Oxidative Stress, Anthocyanins

I. INTRODUCTION

Cadmium (Cd) is a toxic metal to which the general population is exposed via cigarette smoke, industrial products such as batteries, paints, etc and through vegetables and other agricultural products [1, 2]. Cd's toxicity is aggravated by its relatively long half-life and its ability to accumulate in vital human tissues like kidney, liver and testis, where it causes damages [3]. The testis is very sensitive to Cd and as such d has been implicated in the rising cases of infertility among industrial workers and in the general population [4-8]. Cd reproductive toxicity is manifested via Cd-induced oxidative stress and is seen in reduction of testis weight and histopathological lesions leading to reduced sperm counts and impaired sperm motility [9]. Cd being a known endocrine disruptor disrupts the normal function of reproductive hormones which produce and maintain the secondary sexual characteristics of the male and also stimulate sperm production [10,11].

Since Cd's reproductive toxicity is mediated through oxidative stress via increase in reactive oxygen species is, administration of vitamins, lipids, flavonoids and other molecules with antioxidant properties have been shown to ameliorate Cd-induced testicular damage [12-14]. In this regard, researchers have noted the ability of plant pigments such as anthocyanins as antioxidant against Cd toxicity [15-18].

Hibiscus sabdariffa Linn, also known as roselle, is an herbaceous crop native to Central and West Africa and has been reported to contain organic acids, anthocyanins, polysaccharides and flavonoids with potent antioxidant properties [19-20]. Extracts of this plant have long been in use in folk medicine to treat cases of infertility, high blood pressure, and fever [21] though the main active molecules responsible for its effects have not been categorically stated. This study is therefore aimed at exploring the possible changes induced by *H. sabdariffa L* anthocyanins in reproductive hormones of cadmium-exposed rats.

II. MATERIALS AND METHODS

A. Chemicals:

Reagents of analytical grade were used in this study. Cadmium Chloride, methanol, trichloroacetic acid, acetonitrile and sodium chloride were purchased from Lobal Chemic Laboratory Regents and Fine Chemicals, Mumbai – India. Enzyme Immunoassay (EIA) Assay kits for serum testosterone, follicle stimulating hormone (FSH) and LH were products of Biocheck Ltd (UK).

B. Plant Material:

Fresh calyces of *H. Sabdariffa L.* were dried under continuous air-flow maintained at room temperature until constant weight was achieved.

C. Extraction and Purification H. sabdariffa anthocyanins:

Anthocyanins were extracted from *H. sabdariffa calyces* according to the method of Hong and Wrolstad [22] as described by Orororo et al., [23].

D. Experimental animals *and* Experimental Design:

Twenty four (24) adult male wistar rats (185±5.2g) were randomly divided into four treatment groups and where treated for 15 days: Group **A**: control, **B**: Cd alone, 3mg/kg b wt, **C**: HSA alone, 3mg/kg b wt, Group **D**: HSA Pre-CD: HSA (3g/ kg b wt for ten consecutive days) and Cd (3mg/ kg b wt) for five days. At the end of the treatment period, the animals were sacrificed by cervical dislocation and blood samples were obtained by cardiac puncture into heparinized bottles and centrifuged at 3000g for 10 min. Sera collected was stored frozen until used for biochemical analysis.

E. Biochemical Assays:

Serum FSH, LH and testosterone were measured based on the principle of solid phase enzyme-linked immunosorbent assay (ELISA) using Biochek test kit.

F. Analysis of Data*:*

Results obtained in the study were presented as Mean ± SD. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software. The one-way analysis of variance (ANOVA) was utilized in comparing the degree of significance of different parameters estimated and the difference between mean were considered to be significant at p< 0.05

III. RESULTS AND DISCUSSION

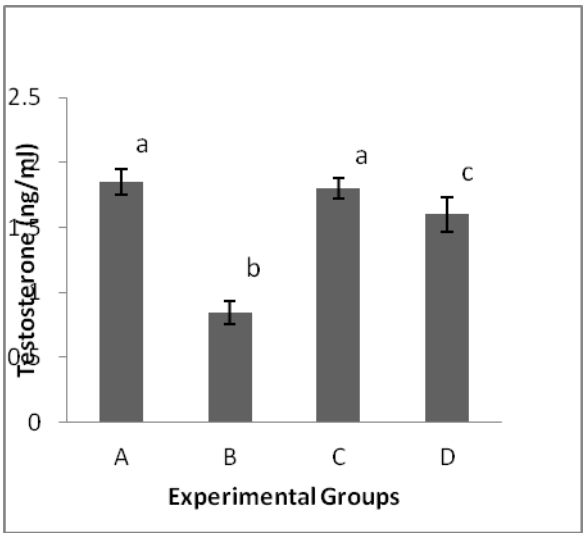

Fig.1: *H. sabdariffa* anthocyanins-induced changes in serum testosterone of Cd-exposed rats. Groups: A (Control), **B** (Cd), **C** (Anthocyanin), **D** (Anthocyanin Pre-Cd). Values with different alphabetic superscripts differ significantly (P<0.05)

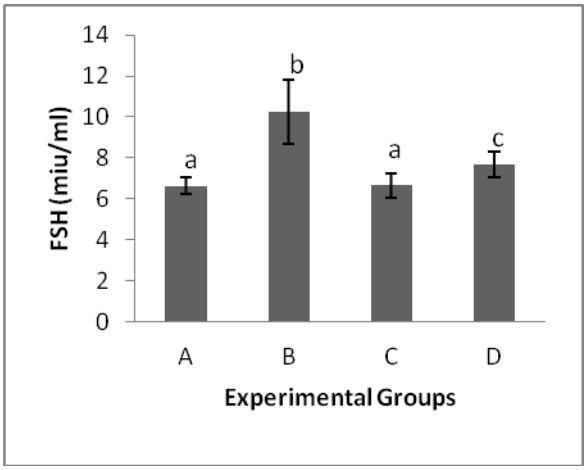

Fig.2: *H. sabdariffa* anthocyanins-induced changes in serum FSH of Cd-exposed rats. Groups: A (Control), **B** (Cd), **C** (Anthocyanin), **D** (Anthocyanin Pre-Cd). Values with different alphabetic superscripts differ significantly (P<0.05)

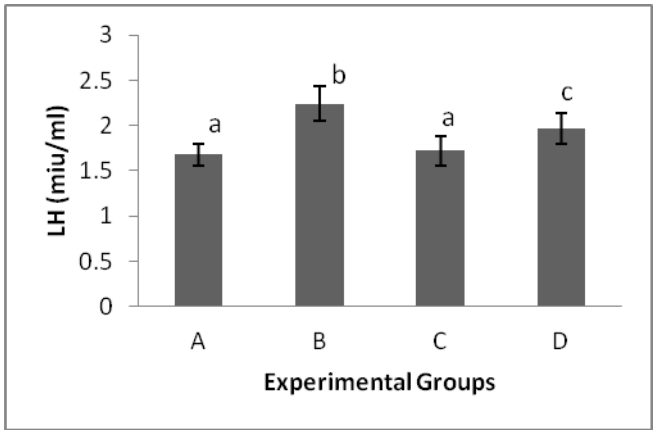

Fig.3: *H. sabdariffa* anthocyanins-induced changes in serum LH of Cd-exposed rats. Groups: A (Control), **B** (Cd), **C** (Anthocyanin), **D** (Anthocyanin Pre-Cd). Values with different alphabetic superscripts differ significantly (P<0.05)

H. sabdariffa anthocyanins-induced changes in the hormones of Cd-exposed rats is shown in Fig 1-3. Comparison of the serum testosterone level of the control group with that of the Cd alone group showed a significant reduction (P<0.05) in the level of serum testosterone. The results indicated that Cd administration lowered the level of serum testosterone and also showed that the administration of *H. sabdariffa* anthocyanin before Cd exposure significantly reverted the decrease in serum testosterone. Conversely, there was significant increase (P<0.05) in the level of serum follicle stimulating hormone (FSH) after cadmium administration (Group C) compared to animals in the control group (Group A) and those maintained on only *H. sabdariffa* anthocyanin alone . This decrease in serum follicle stimulating hormone (FSH) occasioned by Cd exposure was significantly (P<0.05) reversed by the administration of *H. sabdariffa* anthocyanin before Cd exposure. This was also observed for Luteinizing hormone (fig. 3), and points to the antioxidant potentials of HSA.

Increase in plasma levels of FSH after cadmium administration observed in this study could be attributed to the bioaccumulation of cadmium in the testis and is consistent with the findings of [24] who noted that through its effect on Sertoli cell activity, Cd decreases inhibin (the main inhibitory signal for FSH secretion) synthesis and release thereby causing increase in the plasma levels of FSH. This is in line with the changes observed by [25] and [26]. FSH, a pituitary glycoprotein, affects the sertoli cells to stimulate and initiate germ cell number and also enhance the production of androgens by the Leydig cells [27]). FSH also plays a significant role in the maturational stages of spermatozoa. Orisakwe et al., [28], Brian et al., [29] and Omotuyi et al., [30] previously reported that extracts of *H. sabdariffa* calyces are rich in phytoestrogens and thus possess estrogenic effects which reduces serum testosterone levels. Interesting, this was not observed in this study and it can be attributed to the difference in the extracts. Aqueous extracts used in those studies probably content other bio molecules responsible for the estrogenic effects unlike the purified HSA used in this study. In consonance with the results of this study is the work of Fadairo et al., [31] who reported the protective effect of whole and anthocyanin-free aqueous extracts of *Hibiscus sabdariffa L*. on Cadmium-induced changes in serum reproductive hormones of Wistar Rats. Again, Ajiboye et al., [32] attributed this effect of Roselle calyx extract to its anthocyanins, a claim supported by the results of this study.

IV. CONCLUSION

This study is a confirmation of the reproductive toxicity of Cd as all the hormones assayed were significantly altered by exposure to Cd. However, the pre-treatment of Cd-exposed rats with HSA significantly normalized reproductive hormones. This effect of HSA is not unconnected with the reported antioxidant capacities of anthocyanins. The results of this study also gives credence to prominent role attributed to HSA in the antioxidant prowess of *H. Sabdarriffa* extracts.

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