Original Research Article

Changes in reproductive hormones levels following administration of hydroethanolic extract of *Azanza garckeana* fruit in cadmium chloride exposed female Wistar rats

Abstract

There have been many claims of aphrodisiac and other beneficial attributes of *Azanza garckeana* fruit by both tradomedicinal practitioners and some early scientific investigations; the present study, thus, set out to investigate the changes in reproductive hormones following administration of hydroethanolic extract of *azanza garckeana* fruit (HEAGF) in cadmium chloride exposed female Wistar rats. Thirty five (35) female Wistar rats weighing 230±10 grams were divided into 7 groups of 5 rats each. Group 1 served as negative control that received 1ml of distilled water; Group 2 served as positive control and received oral dose of 10mg/kg body weight of $CdCl_2$ (or Cd); Group 3 had Clomiphene citrate (0.3mg/ml) + Cd; Group 4 received Vitamin E (0.3mg/ml) + Cd; Group 5 received 250mg/kg HEAGF+ Cd; Group 6 received 500mg/kg HEAGF+ Cd and Group 7received 1000mg/kg HEAGF+ Cd. All treatments were done orally and daily for 28 days. Blood samples were collected, and serum levels of reproductive hormones including estrogen, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured using ELISA and spectrophotometric technique. The results showed that CdCl2 exposure significantly (P<0.05) decreased FSH level but non-significantly (P>0.05) decreased LH level while increasing oestrogen and progesterone levels compared to the negative control group. However, administration of HEAGF, especially the 250mg/kg bw dose most appreciably restored the hormonal balance towards normal levels. In conclusion, although, the findings of the present study highlights the potential therapeutic value of HEAGF in improving the adverse effects of an environmental toxin—CdCl2 on female reproductive health indicators, further studies may be necessary to elucidate the underlying mechanisms of action prior to its clinical exploration and possible recommendation for supplementation in reproductive disorders associated with a heavy metal toxicity.

Keywords: *Hydroethanolic extract of Azanza garckeana fruit (HEAGF), Cadmium chloride, Female Wistar rats, Female reproductive hormones,*

Introduction

Societal civilization and advancement have brought with them a number of challenges including adverse health conditions and others [1, 2]. For instance, infertility, as a major reproductive health concern of many couples [3, 4], has many of its cases linked to the adverse effects of endocrine disruptors [5]. Reports have it that extensive exploration of the environment has predisposed man to many harmful scenarios (Bindraban et al., 2020) [6]; for instance, the food industry uses numerous chemicals that enter the food chain and have an immediate negative impact on human health [7, 8]. The endocrine disruptive tendencies of many toxic chemical or heavy metals have proven to interfere with normal hormonal actions, metabolism and biosynthesis. Such may result in a deviation from the normal homeostasis of hormones [5].

Certain endocrine disruptors, such as heavy metals, most synthetic chemicals, and preservatives, are strongly linked to conditions like polycystic ovary syndrome, endometriosis, irregular menstrual cycles, and disruptions in steroidogenesis and ovarian follicle development, which are positively correlated with female infertility [5, 9, 10].

Consequently, while the orthodox intervention in the management of infertility is yet to resolve many such difficult conditions, the benefits of resorting to complementary and alternative medicaments in the the management of infertility have been claimed by several scholars [11, 12, 13]. In line with the foregoing, some preliminary investigations have pointed at the possible aphrodisiac and fecundity potentials of *Azanza garckeana* fruit pulp in mammalian models [14,15, 16].

Azanza garckeana is a medicinal plant that is widely found throughout Africa; it is also referred to as the African chewing stick or snot apple [17, 18]. Traditional medicine has made use of several HEAGF components due to their claimed hepatoprotective, anti-inflammatory, and antioxidant qualities [19]. However, there is still much to understand about HEAGF's possible defence against reproductive toxicity [20, 21, 22] especially environmental insults to the system.

For example, the environmental contamination with cadmium chloride (CdCl2), a hazardous heavy metal, can adversely affect the health of both humans and animals [23]. Ingestion of tainted food and water, exposure to unsafe industrial operations, and tobacco use can all lead to CdCl2 contamination. More so, exposure to CdCl2 has been shown to cause a number of harmful health effects, including toxicity to the reproductive system [24, 25]. Toxic levels of CdCl2 in female mammals has been demonstrated to disturb the delicate stability of reproductive hormones, including FSH, progesterone, oestrogen, and LH, consequently impairing normal reproductive function [26, 27].

Thus, the present study set to examine the changes in reproductive hormones following administration of hydroethanolic extract of *azanza garckeana* fruit in cadmium chloride exposed female wistar rats; thereby determining the fertility/fecundity potentials of the HEAGF pulp.

Materials and Methods

Collection and identification of Azanza garckeana Fruit Extract

Fresh fruits of HEAGF were obtained from a local market in Tula, Gombe State, Nigeria and authenticated by Dr. Ekeke, Chemezie, a plant taxonomist at the University of Port Harcourt, Nigeria. Samples for herbarium voucher were deposited and voucher number UPH/P/414, obtained.

Extraction and preparation of plant extract

The fruits were properly washed, sliced, and air-dried under shade for fourteen days. As the sliced HEAGF got dried it was then pulverized with the help of a mechanical grinder. Hydroethanolic extract of HEAGF was prepared by macerating the powdered fruits in a mixture of ethanol and water (70:30) for 72 hours. The extract was filtered using Whatman paper 1, and the solvent was evaporated at 45°C using a rotary evaporator. The obtained crude extract was stored at 4°C until when used.

Animal Acquisition and Handling

Thirty five (35) female Wistar rats weighing 230±10 grams were procured from the Animal House of the Department of Pharmacology, Faculty of Basic Medical sciences, University of Port Harcourt. The study animals were housed in wire-gauzed plastic cages with saw-dust beddings and maintained at room temperature under the 12 hour light/dark cycle. The animals were acclimatized to their new handling and accommodation for at least seven (7) days prior to commencement of experimentations. The study animals were handled as recommended by the US National Institute of Health (NIH) guidelines for care/use of laboratory animals in analytical studies.

Experimental Protocol

Thirty five (35) female Wistar rats were randomly divided into seven groups of five rats per group. The distribution is as follows:

Group 1: Negative control that received 1ml of distilled water
Group 2: Positive control that received $CdCl_2$ (or Cd)
Group 3: that received Clomiphene citrate (0.3mg/ml) + Cd
Group 4: that received Vitamin E (0.3mg/ml) + Cd
Group 5: that received 250mg/kg HEAGF+ Cd
Group 6: that received 500mg/kg HEAGF+ Cd
Group 7: that received 1000mg/kg HEAGF+ Cd

Sample Collection and Hormonal Assays

After twenty (28) consecutive days of treatments, blood samples were collected from the study models under light anesthesia via cardiac puncture and put into well labeled plain sample bottles. Thereafter, the samples were centrifuged at 3000 rpm for 15 minutes, and the serum was separated and stored at about -85°C prior to further analysis. The serum levels of estrogen, progesterone, FSH, and LH were subsequently determined using the spectrophotometric detection technique and the enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions [28, 29, 30].

Statistical Analysis

The data were presented as mean ± standard error of the mean (SEM) and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. The statistical significance was set at $p < 0.05$.

Ethical Approval

Approval for the current study was obtained from the Central Research Ethics Committee of the University of Port Harcourt with reference number: UPH/CEREMAD/REC/MM89/231, dated June 1, 2023.

Results

The data on Table 1 shows the changes in reproductive hormones following administration of hydroethanolic extract of Azanza garckeana fruit (HEAGF) in cadmium chloride exposed female Wistar rats.

Considering the changes in follicle stimulating hormone (FSH) level, both the positive control group (group 2 treated with $CdCl2$ only) and the rest of the treated groups (groups 3 to 7) indicated markedly ($P<0.05$) reduced levels of FSH when compared to that of the negative control (untreated normal rats). Although not significantly ($P>0.05$), groups 3, 4, 6 and 7 treated with Clomiphen citrate (100mg/kg) + Cd, Vitamin E (0.3mg/ml) + Cd, 500mg/kg HEAGF+ Cd and 1000mg/kg HEAGF+ Cd respectively had reduced levels of FSH when compared to that of the positive control.

Table 1: Changes in Reproductive hormones following administration of hydroethanolic extract of Azanza garckeana fruit (HEAGF) in Cadmium Chloride exposed female Wistar Rats

Groups/Treatment(s)	FSH (mlU/ml)	LH (mlU/ml)	Oestrogen (pg/ml)	Progesterone (ng/ml)
Group 1: Negative control	0.69 ± 0.17	0.51 ± 0.15	72.00 ± 20.33	16.62 ± 5.32
Group 2: Positive control + Cd	0.40 ± 0.07 [a]	0.36 ± 0.08	101.80 ± 20.59	19.18 ± 1.38
Group 3: Clomiphene citrate (100mg/kg) + Cd	0.23 ± 0.04 [a]	0.81 ± 0.09 [a, b]	105.80 ± 2.13 [a]	27.82 ± 3.10 [a]
Group 4: Vitamin E (0.3mg/ml) **+ Cd**	0.19 ± 0.02 [a]	0.88 ± 0.11 [a, b]	94.00 ± 7.26	25.34 ± 1.88
Group 5: 250mg/kg HEAGF+ Cd	0.43 ± 0.08 [a]	1.05 ± 0.05 [a, b]	91.60 ± 5.83	22.18 ± 1.80
Group 6: 500mg/kg HEAGF+ Cd	0.26 ± 0.01 [a]	0.52 ± 0.04 [c, d, e]	82.20 ± 7.99	23.78 ± 1.96
Group 7: 1000mg/kg HEAGF+ Cd	0.26 ± 0.03 [a]	0.71 ± 0.08 [b, e]	78.80 ± 1.77	24.12 ± 1.98

Values represent mean ± SEM, n=5; [a] Significant at p<0.05 when compared to group 1; [b] Significant at p<0.05 when compared to group 2. [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4; [e] Significant at p<0.05 when compared to group 5; [f]Significant at p<0.05 when compared to group 6. FSH (mlU/ml) = Follicle Stimulating Hormone; LH (mlU/ml) = Luteinizing Hormone. HEAGF = Azanza garckeana fruit. Cd= $CdCl_2$.

The level of luteinizing hormone (LH) was found to be lowest in the group 2 rats, but it was only marginal when compared to that of the group 1 rats. Groups 3, 4 and 5 indicated markedly raised levels of LH when compared to those of groups 1, 2 and 6. Group 7 also showed remarkable increase in its LH level when compared to that of group 2 but low with respect to that of group 5.

In view of the changes in oestrogen and progesterone levels, there were marginal elevations in the mean values of groups 2, 4 to 7 when compared to that of group 1. Only the mean oestrogen and progesterone levels of group 3 had marked elevation when compared to that of group 1.

Discussion

Reproductive toxicity due to some environmental toxicants (e.g. heavy metals, like CdCl2) and other causes are frequently associated with alterations in hormonal balance (including estrogen, progesterone, FSH and LH) [31, 32]. The diagnosis and management of dysfunctional hormonal levels in infertility may be quite challenging [33, 34]; but the synergistic and natural mechanism of modulating dysfunctional hormonal levels by herbs have been noted by some earlier investigations [35, 36].

The present study evaluated the changes in reproductive hormones following administration of hydroethanolic extract of HEAGF in cadmium chloride exposed female Wistar rats and made some remarkable findings which are discussed in the subsequent paragraphs.

The present study recorded markedly reduced FSH levels in all Cd treated animals with respect to that of the control group. In fact, these decreases were much more in the Cd co-treated with vitamin E, clomiphene, and the medium/high doses of HEAGF. Only the low dose HEAGF (250mg/kg HEAGF+ Cd) treated group had appreciable elevation in its mean FSH level. While the foregoing outcome of the present study is in line with some earlier reports [14, 32] it has specifically shown that low/moderate dose of the HEAGF may have potent ameliorative effect on depressed FSH levels. Such attribute may be connected to the abundant constituents of beneficial phytochemicals [37, 38]. Surprising and worthy of note from this finding is that, the low dose of HEAGF (250mg/kg) better improved the Cd altered FSH level when compared to those of Clomiphene + Cd and Vitamin E + Cd treated groups. This finding thus indicates that higher doses of hydroethanolic extract of HEAGF may not elicit the supposed ameliorative effect on decreased FSH level.

The result of the changes in the level of LH indicated marginal decrease in the Cd-only intoxicated group (group 2) when compared to that of the control group; all other groups co-treated with Cd + vitamin E, Cd + clomiphene, and the Cd + medium/high doses of HEAGF, respectively, showed significant elevation in their LH levels with respect to those of groups 1and 2. The low dose HEAGF (250mg/kg HEAGF+ Cd) treated group had the most marked elevation in its mean FSH level. This outcome on LH level is similar to the earlier findings of Ahmad et al., [39] and Nurudeen et al., [15]. Interestingly, the lowest dose of the hydroethanolic HEAGF treated group (Group 5), had the most raised mean level of LH. The boosting of depleted LH level in the low dose of HEAGF treated rats could possibly imply enhancement of the production of sex steroid like progesterone; such is said to be possible by upregulating the expression of the enzymes that facilitate progesterone production [40].

In another finding of the present study, it was seen that the changes in oestrogen and progesterone levels had marginal elevations in the mean values of groups 2, 4 to 7 when compared to that of group 1. Only the mean oestrogen and progesterone levels of group 3 had marked elevation when compared to that of group 1. Although the current finding corroborates with some preliminary reports [14, 15], but particularly revealed that increasing doses of the HEAGF seem to have exerted decreasing levels of oestrogen in its dysfunctional high level. And on the other hand, there were increasing levels of progesterone with increasing doses of HEAGF. These effects may be attributed to the phytochemical constituents present in HEAGF, such as flavonoids, alkaloids, and phenolic compounds, which have been reported to possess estrogenic and progestogenic properties [19, 38, 41]. Additionally, HEAGF may have also exerted antioxidant effects [38, 42], thereby indicating a protective attribute on the ovaries from oxidative damage possibly induced by CdCl2 intoxication. Considering the foregoing findings of the present study, the ethnobotanical application of the different portions of the *Azanza garckeana* plant in the treatment of infertility and other related conditions may be justified to an extent. However, caused must be exercised as increasing doses of the HEAGF may elicit different and sometimes perhaps unbeneficial effects on the individual female reproductive hormones.

Conclusion

The hydroethanolic extract of *Azanza garckeana* fruit (HEAGF) has shown the possible attributes of protective effects against CdCl2-induced reproductive toxicity in female Wistar rats by restoring the hormonal balance. Of course, the findings of the present study highlight the potential therapeutic value of HEAGF in improving the adverse effects of an environmental toxin—CdCl2 or Cd, on female reproductive health. Further studies may be necessary to clarify the possible underlying mechanisms of action of the HEAGF and its actual effects on the respective reproductive hormones in lower mammals prior to its clinical exploration and possible recommendation for supplementation in reproductive disorders associated with a heavy metal toxicity.

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