



Evaluation of the Reproductive Potentials of *Azanza Garckeana* Fruit Extract on Lead Treated Male Wistar Rats

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Abstract

Reproduction is an important biological trait to produce new individual organisms and is fundamental for the life of an individual as well as the survival and development of the species. The current study evaluated the effects of Hydro-methanolic fruit extract of *Azanza garckeana* on reproductive functions in male Wistar rats. Twenty (20) Wistar rats divided into 4 groups of 5 rats each were used for the study. Group 1 served as negative control and received only water and feed; group 2 received 5mg/kg of lead acetate only, while groups 3 and 4 were administered with 200mg/kg and 400mg/kg of Hydro-methanolic fruit extract of *Azanza garckeana* respectively for a period of 28 days. At the end, the animals were anaesthetized with chlorofoam and blood was collected through cardiac puncture for hormone analysis. Semen was obtained for sperm analysis. The result showed that follicle stimulating hormone and luteinizing hormone and testosterone decreased significantly ($p < 0.05$) in the positive control (lead only) group when compared to the negative control. There was a significant increase in testosterone level in group 3 (lead + 200 mg/kg) and group 4 (lead + 400 mg/kg) when compared to the lead only group. The sperm parameters including viability, normal morphology, actively motile and count were decreased in the lead only group, but significantly increased in the groups that received *Azanza garckeana* extract. In conclusion, extracts of *Azanza garckeana* improved reproductive parameters in male Wistar rats with lead induced reproductive toxicity.

Keywords: *Azanza garckeana*, hydromethanolic, Wistar rats, reproductive functions.

Introduction

It is an established fact that over the past decades, infertility in couples has posed enormous challenge to reproductive health. Although, infertility has a global trend, the pattern in

developing countries including the sub-Saharan Africa is quite disturbing. These challenges have prompted many studies by scientists from different fields of life, with the sole purpose of increasing search for fertility enhancing products,

substances or materials particularly among the reproductive age. The outcome is encouraging as many drugs that can improve fertility, particularly in men has been produced. However, use of these orthodox medicines are accompanied with some challenges such as side effects of drugs, high cost of drugs and non-availability of drugs (Luck *et al.*, 1995; Dietrich *et al.*, 2003). This situation calls for improved effort into search for medicinal plants with fertility enhancing properties.

Azanza garckeana is a readily available, cheap medicinal plant with little or no adverse effects which reportedly contains specific plant pigments with anti oxidative ability such as vitamins C, E, A, carotenoids - lutein (Siddhuraju and Becker, 2003; Aslam *et al.*, 2005). The fruits of this plant were reported in some studies to have potentials of improving fertility in female experimental Wistar rats (Dietrich *et al.*, 2003). In males, *Azanza garckeana* is consumed traditionally as a remedy for poor libido but its effect on male fertility is not well documented.

Lead acetate has been shown to have some adverse effects on fertility potentials of albino Wistar rats by reducing weight and distorting the histology of some reproductive organs (Iranloye and Bolarinwa, 2009); as well as exerting toxic effects on body tissues including the testis (Pounds, 1985; Antilla, 1990; Sugiyama, 1992).

Lead has been shown to cause reproductive/testicular toxicity by inducing oxidative stress and *Azanza garckeana* may influence effects of oxidative stress in tissue of rats.

The aim of this study is to evaluate the effects of *Azanza garckeana* on lead induced reproductive toxicity in male Wistar rats.

Materials and Methods

This study was done in the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria.

Collection and identification of plant materials

Fresh fruits of *Azanza garckeana* were purchased from a local market in Woji community in

Obio/Akpor Local Government Area of Rivers State, Nigeria. They were identified and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt, with the reference number: UPH/V/2,213.

Extraction of plant extract

Extraction was carried out at the department of Pharmacy and Pharmacognosy, University of Port Harcourt, Rivers State, Nigeria. The fresh fruits were rinsed thoroughly in distilled water and dried for 20 days. The dried fruits were grounded to fine powder, using a domestic electric grinder and 750g of the plant material was obtained. Hydromethanol (1:4) was used as the solvent. The filtrate were pulled together and lyophilized using a freeze dryer. The yield of the hydromethanolic fruits extracts of *Azanza garckeana* was 18.22% (w/w). The lyophilised extract was stored air tight and kept in the dark until used.

Experimental Animals

Twenty (20) male Wistar rats were used for this study. The animals were procured from animal house, Department of Human Physiology, University of Port Harcourt, Rivers State. The animals were housed in a spacious and well ventilated cage with suitable temperature, relative humidity under 12 hour light and dark cycle. They were acclimatized for two (2) weeks and allowed access to feeds and water *ad libitum*.

All animals received human care according to the criteria outlined in the guide for the care and the use of Laboratory animals prepared by the National Academy of Science and published by the National Institute of Health. The ethical regulations in accordance with National and Institutional guidelines for the protection of animals' welfare were strictly adhered to during the experiments PHS (1996).

Experimental Design

A total of twenty (20) Wistar rats were used for this study, which were divided into 4 groups of 5 rats each. Group 1 served as control and received

only water and feed. Group 2 was administered with only 5mg/kg of lead. Groups 3 and 4 were administered with 200mg/kg and 400mg/kg of hydro-methanolic fruit extract of *Azanza garckeana* +5mg/kg of lead respectively.

The administrations lasted for 28 days after which the animals were sacrificed and the blood samples and semen collected for hormone assay and sperm analysis.

Hormone Analysis

The assay for testosterone and prolactin measured in ng/ml, luteinizing hormone and follicle stimulating hormone measured in m/μ/ml were done in accordance with established protocols and in complete observance with manufacturers' guidelines (Tietz,1986; Layman,1997)

Sperm Analysis

The epididymis was lacerated and semen was collected, emulsified with 0.5% Eosin and examined using x10 & x 40 objective lens. Also

10-12 fields was examined to check for viable cells which is the % of stained cells against the unstained cells. Also 10-12 field was examined to check for percentage of normal cells as against the abnormal ones. The same was done for actively motile cells. The sperm count was done using counting chamber and 1:20 dilution of the semen was done using normal saline. The chamber was assembled and filled with the diluents. The counting of the 4 x 16 squares was done and then multiplied by100,000.

Statistical Analysis

All statistical analysis were done using Statistical Package for Social Science (SPSS), version 20.0 software. The data were analyzed using One way Analysis of Variance (ANOVA) and Dunnett's Post hoc (multiple comparison) was used to decide the level of significance between control and test groups. The difference was considered significant at (P<0.05). The result was expressed in tables as mean ± SEM.

Results

The results are presented in tables 1-4.

Table 1: Effect of extract of *Azanza garckeana* on FSH and LH levels

Groups	FSH (m/μ/ml)	Relative change (%)	LH (m/μ/ml)	Relative change (%)
Negative control	1.15±0.68	0	1.30±1.02	0
Positive control (Lead 5mg/kg)	0.19±0.15*	-83.48	0.34±0.29*	-73.85
200 mg/kg extract + (Lead 5mg/kg)	0.41±0.28*	-64.35	0.45±0.39*	-65.38
400 mg/kg extract + (Lead 5mg/kg)	0.28±0.23*	-75.65	0.42±0.25*	-67.69

Values expressed as mean±SEM. n=5. * means values are statistically significant when compared to the negative control.

Table 2: Effect of extract of *Azanza garckeana* on Testosterone and Prolactin levels

Groups	Testosterone (ng/ml)	Relative change (%)	Prolactin (ng/ml)	Relative change (%)
Negative control	4.46±3.13	0	2.72±1.72	0
Positive control (Lead 5mg/kg)	1.35±0.69*	-69.73	0.34±0.19*	-87.50
200 mg/kg extract + (Lead 5mg/kg)	1.64±0.45*	-63.23	0.54±0.48*	-80.15
400 mg/kg extract + (Lead 5mg/kg)	2.78±2.28*#	-37.67	0.21±0.12*	-92.28

Values expressed as mean±SEM. n=5. */# means values are statistically significant when compared to the negative and the positive controls respectively.

Table 3: Effect of extract of *Azanza garckeana* on sperm parameters

Groups	Viable Cells (%)	Normal Morphology (%)	Actively motile cells (%)	Sperm count (x 10 ⁶ /ml)
Negative control	82.00±2.55	78.00±2.54	76.00±1.87 [#]	120.00±1.07
Positive control (Lead 5mg/kg)	63.00±2.55 [*]	64.00±2.45 [*]	54.00±4.85 [*]	50.00±1.40 [*]
200 mg/kg extract + (Lead 5mg/kg)	82.00±2.00 [#]	81.00±1.00 [#]	76.00±2.45 [#]	128.00±4.90 [#]
400 mg/kg extract + (Lead 5mg/kg)	88.00±2.00 [#]	89.00±2.45 ^{*#}	86.00±1.87 [#]	164.00±7.48 ^{*#}

Values expressed as mean±SEM. n=5. */# means values are statistically significant when compared to the negative and the positive controls respectively.

Table 4: Effect of extract of *Azanza garckeana* on weights of testes and epididymis

Groups	Testicular weight	Relative change (%)	Epididymal weight	Relative change (%)
Negative control	1.22±0.66	0	0.24±0.24	0
Positive control (Lead 5mg/kg)	1.10±0.55	-9.84	0.14±0.40 [*]	-41.67
200 mg/kg extract + (Lead 5mg/kg)	1.14±0.27	-6.56	0.48±0.38 ^{*#}	100
400 mg/kg extract + (Lead 5mg/kg)	1.40±0.71	14.75	0.36±0.51 ^{*#}	50

Values expressed as mean±SEM. n=5. */# means values are statistically significant when compared to the negative and the positive controls respectively.

Discussion

This study evaluated the reproductive potentials of hydromethanolic fruit extract of *Azanza gackeana* on some reproductive functions in male Wistar rats administered with lead. The hormone measurements indicated that follicle stimulating hormone and luteinizing hormone were significantly decreased in the positive control group that received only lead and in those that received both extracts and lead when compared to the negative control. However, the extracts caused a non-significant increase in both hormones when compared to the lead only (positive control) group. Testosterone level was significantly reduced in the groups that received lead compared to the negative control. However, testosterone level was significantly increased in the group that received higher dose (400mg/kg) of the extract and lead when compared to the lead only group (positive control). These results are in agreement with those reported by El-Sheikh *et al.*, (2016), who revealed an increase in the level of

serum testosterone after administration of *Azanza garckeana* fruit extract. The increased testosterone level acting through a negative feedback effect in the hypothalamic hypophysial gonadal axis, may have led to a non significant change in luteinizing hormone level, as a result of the interaction between luteinizing hormone and testosterone. Testosterone synthesized and produced by testicular interstitial cells is controlled via a feedback mechanism under the hypothalamic-pituitary-gonadal axis control (Chang *et al.*, 2014). Luteinizing hormone binds to its receptor in the testicular interstitial cells to promote testosterone synthesis and subsequent elevation in serum (Huang *et al.*, 2018). The result also show that the serum prolactin level was significantly reduced in the groups that received lead compared to the negative control, however, the prolactin level was not significantly altered in the groups that received lead and extract of *Azanza garckeana* compared to the positive control (lead only group). Similarly, the weight of testes was

not significantly affected in all the groups but epididymal weights were reduced significantly in the groups that received lead compared to the negative control, but the weights were significantly increased in the groups administered with lead and extract of *Azanza garckeana* compared to the positive control.

Evaluation of several semen parameters, including sperm count and sperm motility are essential for proper assessment of male fertility (Komiya *et al.*, 2013). These sperm parameters may reflect any physical or chemical alterations affecting testicular tissue (Atli *et al.*, 2016). Our result showed that all the sperm parameters analysed in this study including percentages of viable sperm cells, sperm cells with normal morphology, actively motile sperm cells and sperm count were significantly decreased in the positive control (lead only) group when compared to the negative control. However, all sperm parameters were significantly increased in the groups that received lead and extract of *Azanza garckeana* compared to the positive control (lead only group). Even so, some of the parameters had values exceeding the negative control value suggesting that the extract of *Azanza garckeana* had tremendous influence in improving sperm parameters in male rats with lead-induced reproductive toxicity. Reproductive toxicity in male rats occasioned by lead usually arise due to damaged testicular tissue structure, abnormal formation and maturation of sperms and decline in semen quality and quantity (Huang *et al.*, 2018). Our findings which indicated a decrease in normal morphology, sperm motility and count induced by lead as well as a significant improvement in testicular functions upon concurrent administration of lead and *Azanza garckeana* agreed with previous report by Priyadarshani and Varma (2014), who stated that an improvement in semen parameters (count, motility, and morphology) occurred after administration of *Azanza garckeana* fruit extract. These observed actions of the extract may be attributed to the reportedly rich antioxidant's characteristics of *Azanza garckeana*, which may

have influenced the epididymal antioxidant system and caused improvement in the spermatogenic process. Following administration with *Azanza garckeana* or isolated phytochemicals, several studies showed improved levels of detoxication biomarkers and antioxidant enzymes (Faizi *et al.*, 1994; Ashok and Pari, 2003).

Furthermore, the ameliorative effect of *Azanza garckeana* extract on sperm parameters which has been attributed to its effect on the antioxidant enzyme system favors the reproductive process and also enhances spermatogenesis (D'cruz and Mathur, 2005). Metal induced toxicity is widely reported in literatures and has been attributed to oxidative stress (Pounds 1985; De-Flora and Wetherhahn 1989). Siddhuraju and Becker (2003) reported that *Azanza garckeana* fruit contain certain antioxidant and phenolic compounds that helps protect the testis against adverse morphologic, spermatogenic and oxidative changes brought about by toxic substances. The toxic actions of metals has been shown to be oxidative in nature (Antilla 1990 ; Leonard *et al.* 2004). It is noteworthy that *Azanza garckeana* fruit may have been able to attenuate lead toxicity due to its antioxidative potential. In a report (De-Cruz, 2005), it was shown that the sperm cytoplasm contained very low concentrations of free radical sweeping enzymes. A possible increase in the antioxidant enzyme activity by *Azanza garckeana* fruit extract treatment favored reproductive potentials in male Wistar rats.

Conclusion

There was a decline in levels of some reproductive parameters due to lead induced toxicity in male Wistar rats. However, administration of hydromethanolic fruit extract of *Azanza garckeana* ameliorated the negative impact of lead on testosterone and sperm parameters including viability, morphology, motility and count in male Wistar rats.

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