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Improvement in some Reproductive Parameters of Male Wistar Rats Administered with Leaf Extract of *Eugenia uniflora*

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Abstract **Original Research Article**

This study was designed to evaluate the effect of hydro-methanol leaf extract of *Eugenia uniflora* on lead-induced reproductive toxicity in male Wistar rats. The male rats used for the study were divided into 5 groups of 6 animals each. Group I was the negative control group which received no treatment. Group II was the positive control which received 5 mg/kg BW Lead daily, while Group III received 5 mg/kg BW of Lead and 200mg/kg BW of extract., Group IV received 5 mg/kg BW of Lead and 400mg/kg BW of extract and group V received 5 mg/kg BW of Lead and 800mg/kg BW of extract. The administration was by oral gavage once daily for 30 days. At the end, animals were sacrificed under light chloroform anesthesia. Blood samples were collected into plain bottles for hormonal assay. The epididymis was located with a small incision on the abdomen and semen sample was collected for analysis of sperm parameters. The data was analysed by applying one-way analysis of variance (ANOVA) using the SPSS version 23 software. Results of this study showed that extract of *Eugenia uniflora* significantly increased the level of serum testosterone and the percentages of viable sperm cells, sperm cells with normal morphology and actively motile sperm cells as well as, sperm count. The extract ameliorated lead induced reproductive toxicity. The result therefore, show that the extract improves sperm quality and quantity and protects the male reproductive function from the hazardous effect of environmental toxicants such as lead.

Keywords: Eugenia uniflora, Wistar rats, anesthesia, epididymis, sperm.

INTRODUCTION

The use of plants to cure several kinds of human diseases has a long history. Various parts of plants such as leaf, stem, bark, root, etc. are being used to prevent, allay symptoms or revert abnormalities back to normal. Since the practice of "herbal remedies" does not adhere strictly to facts accrued using scientific approaches, orthodox medicine sees "herbal medicines" as an alternative medicine. However, most of the pharmaceutical products currently dispensed by physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis and quinine. Modern medicine today utilizes active compounds isolated from higher plants, and about 80% of these active ingredients indicate a positive correlation between their modern therapeutic use and the traditional uses (Sakar *et al.*, 2015).

Eugenia uniflora, a member of the Myrtaceae family, is primarily found in South America's tropical and subtropical regions (Heywood *et al.*, 2007; Wilson, 2011). *Eugenia uniflora*, also known as Pitanga cherry, Brazilian cherry, Surinam cherry, or South cherry, is a South American native that can be found in Brazil, Paraguay, Uruguay, and Argentina (Consolini and Sarubbio, 2002), as well as the Caribbean islands, China, India, Australia, Egypt, and Nigeria (Kanazawa *et al.*, 2000). It is a semi-deciduous shrubby tree with edible, cherry-like fruits that, along with the leaves,

have been regarded as a putative candidate in folk medicine for the management of various ailments because they possess a wide range of medicinal properties, including radical scavenging activities, anti-inflammatory, anti-aging, antiallergic, hypolipidemic, hypoglycemic, anticancer, and cytotoxic properties (Alves, 2008; Amorim *et al.*, 2009; Dartora *et al.*, 2011; Rattmann *et al.*, 2012; Saravanamuttu, 2012; Samy *et al.*, 2014). All of these activities and properties are due to the presence of many phytochemicals in the leaves and in the plant itself, such as tannins, flavonoids, triterpenoids, and alkaloids (Onwudiwe *et al.*, 2010; Bakr *et al.*, 2017), as flavonoids have been identified to be involved in the anti-inflammatory and antioxidant properties of plant extracts (Onwudiwe *et al.*, 2010; Bakr *et al.*, 2017; AbdJalil *et al.*, 2012).

Male infertility, which is regarded one of the most serious challenges confronting married couples, is on the rise around the world, with an estimated prevalence rate of 10–15% (Krausz, 2000). This is due to a number of causes, including exposure to environmental toxins and xenobiotics, both of which have been proven to have negative effects on male reproductive function (Petrelli & Mantovani, 2002), resulting in a gradual deterioration in male fertility and overall reproductive health (Sikka & Wang, 2008; Wong & Cheng, 2011).

Lead (Pb), is an abundant heavy toxic metal that is absorbed into the blood to bind to erythrocytes and then distributed to other tissues where it causes a wide range of toxic physiological, biochemical, and histological effects. It is a toxicant with reported adverse effects on male reproductive function (Duruibe *et al.*, 2007; Okediran *et al.*, 2017). Lead (Pb) is a poisonous heavy metal that is widely distributed in the environment and is one of the metal contaminants (Herman *et al.*, 2007). Lead poisoning affects a variety of important functions, including reproduction (Biswas and Ghosh, 2004). Lead poisoning can induce developmental neurotoxicity, reproductive dysfunction, and toxicity to the blood, kidneys, and endocrine systems, among other things (Sanborn *et al.*, 2002). Lead poisoning has serious reproductive implications, and practically every compartment of the reproductive system is sensitive to Lead stress (Marchlewicz *et al.*, 2007). The aim of this study is to evaluate the effect of hydro-methanol leaf extract of *Eugenia uniflora* on lead-induced toxicity on some male reproductive parameters.

MATERIALS AND METHODS

Animal Models

A total of 30 adult male Wistar rats were bred in the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, Rivers State University, Nigeria. The animals had an average weight of 130-150g. They were placed in standard cages and acclimatized in two weeks while maintaining them in environmental conditions with proper ventilation. Generally, the procedures conformed to the established principles for the care and use of laboratory animals published by the National Institute of Health, USA (National Institutes for Health, 1985). Appropriate institutional approval was obtained for this study.

Preparation of Plant Extract

The *Eugenia uniflora* leaves used for this study was obtained from Adanta-Isiokpo community in Ikwerre Local Government Area of Rivers state, Nigeria and subsequently identified by the taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The plant's voucher specimen was deposited at the herbarium. The leaves were dried in the air for two weeks before being pulverized into powder. The powdered material was dissolved for 72 hours in a maceration jar containing 80% methanol and 20% water. For the three days it was left to sit, the mixture was agitated three times a day. The substance was filtered with a white handkerchief and re-filtered with filter paper at the conclusion of the maceration period to obtain a clear filtrate. A rotary evaporator was used to concentrate the filtrate at an optimal temperature (40-50^{o}C). The extract-containing filtrate was placed into an evaporating dish and dried on a 45^{o}C water bath until it dried into a pastry form.

Experimental Design/Procedure

This study was designed to evaluate the effect of hydro-methanol leaf extract of *Eugenia uniflora* on lead-induced toxicity on some male reproductive parameters. The male rats used for the study were divided into 5 groups of 6 animals each as; Group I was the negative control group which received no treatment. Group II was the positive control which received 5 mg/kg BW Lead daily, while Groups III received 5 mg/kg BW of Lead and 200mg/kg BW of extract., Group IV received 5 mg/kg BW of Lead and 400mg/kg BW of extract and group V received 5 mg/kg BW of Lead and 800mg/kg BW of extract. The administration was by oral gavage once daily while the extract was administered for 30 days. The dose of the extract employed in this investigation was based on Onwudiwe *et al.*, (2010)'s acute toxicity (LD50) study, which found that the acute toxicity (LD50) of the alcohol extract of Eugenia uniflora only occurs at doses greater than 2408.3mg/kg BW. This indicates that the plant extract has a high tolerance and safety level within the provided amounts.

The animals were sacrificed under light chloroform anesthesia at the end of the dosing period. Their blood samples were taken via cardiac puncture and placed in plain bottles for hormonal analysis (FSH, LH and T). The testis and epididymis were weighed, and sperm parameters including sperm motility, sperm count, sperm viability and sperm morphology were analysed in accordance with established methods.

STATISTICAL ANALYSIS

Results are expressed as mean ± standard error of mean (SEM). Significant differences were determined by one-way analysis of variance (ANOVA) using SPSS version 23 (SPSS incorporated, Chicago, Illinois, USA). The differences in values were considered to be statistically significant at $p<0.05$.

RESULT

The result of this study are presented in Tables 1-3.

Table 1: Effect of the hydromethanol extract of *Eugenia uniflora* on testosterone, FSH and LH

Groups	Testosterone ng/ml	FSH m/µ/ml	LH m/µ/ml
I	2.03 ± 0.06	0.42 ± 0.09	0.90 ± 0.41
II	0.76 ± 0.02$^{\#}$	0.50 ± 0.10	0.34 ± 0.05$^{\#}$
III	0.36 ± 0.02$^{\#*}$	0.52 ± 0.13	0.60 ± 0.04
IV	0.48 ± 0.04$^{\#}$	0.88 ± 0.46$^{\#*}$	0.76 ± 0.02
V	1.17 ± 0.03$^{\#*}$	1.06 ± 0.04$^{\#*}$	0.81 ± 0.03

Values are expressed as mean ± SEM, n=6. */ $^{\#}$Significantly different from positive and negative control groups at (P<0.05) respectively

Table 2: Effect of the hydromethanol extract of *Eugenia uniflora* on sperm cell parameters

GROUPS	Sperm Viability (%)	Normal Morphology (%)	Actively Motile (%)	Sperm Count (x10^6/ml)
I	75.00 ± 3.54	78.00 ± 5.00	70.00 ± 4.47	112.00 ± 10.20
II	53.00 ± 2.24	58.00 ± 3.74$^{\#}$	44.00 ± 5.34$^{\#}$	23.20 ± 5.31$^{\#}$
III	63.00 ± 3.00$^{\#}$*	56.00 ± 3.67$^{\#}$	53.00 ± 2.00$^{\#}$	34.00 ± 5.10$^{\#}$
IV	68.00 ± 3.74*	72.00 ± 4.64*	67.00 ± 5.15*	72.00 ± 10.20$^{\#*}$
V	74.00 ± 2.45*	70.00 ± 3.16*	69.00 ± 4.30*	94.00 ± 10.77*

Values are expressed as mean ± SEM, n=6. */$^{\#}$ significantly different from positive and negative control groups at (P<0.05).

Table 3: Effect of the hydromethanol extract of *Eugenia uniflora* on testicular and epididymal weights

Groups	Testis (g)	Epididymis (g)
I	1.12 ± 0.12	0.24 ± 0.07
II	1.30 ± 0.16	0.15 ± 0.04
III	1.27 ± 0.20	0.19 ± 0.04
IV	1.13 ± 0.17	0.14 ± 0.02
V	1.24 ± 0.15	0.17 ± 0.05

Values are expressed as mean ± SEM, n=6

DISCUSSION

The gonadotropic hormones such as Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH) as well as Testosterone (T), are the most important endocrine variables influencing testes function (LH). Testosterone is a crucial regulator of spermatogenesis in seminiferous tubules that is released by Leydig cells in the testes in response to LH activation (Smith and Walker, 2014). The serum reproductive hormone profile, as shown in Table 1, revealed that lead administration resulted in a significant (P<0.05) decline in testosterone and LH levels, whereas co-delivery of the extract with lead resulted in an increase in testosterone level. Amjad *et al*., (2013) similarly revealed lead-induced reductions in the plasma levels of reproductive hormones, whereas Mukherjee and Mukhopadhyang (2009) and Taiwo *et al*., (2010) found no significant changes in testosterone level. These differences in the effects of lead on hormone levels could be due to differences in lead dosage and/or period of time the animals were exposed to lead (Mohsen, 2011), while other theories suggest that the lead-exposed animal is able to adapt to the metal's toxic effects due to a decrease in GnRH mRNA levels thereby leading to no significant change in plasma GnRH and LH levels (Sokol *et al.*, 2002; Mohsen, 2011). The ameliorative action of *Eugenia uniflora* extract against lead toxicity has been described in some plant extracts [Kolawole *et al.*, (2014); Soleimanzadeh *et al.*, (2018, 2020)]. The authors reported increase in reproductive hormone levels when the extracts were co-administered with lead. In the present study, co-administration with lead ameliorated lead induced significant reductions in testosterone level thereby causing significant increase in testosterone and marginal increase in LH level when compared to the lead group.

The sperm cell parameters in this study were depleted upon administration of Lead. These parameters have direct influence on testicular tissues and sperm quality (Wang *et al*., 2008). However, improvements were noted in the percentages of these parameters upon administration of *Eugenia uniflora* extract. These ameliorative effect of the extract on lead induced reproductive toxicity provides evidence to support that the extract could be a potent fertility booster as a result of its ability to improve percentages of viable sperm cells, sperm cells with normal morphology, actively motile sperm cells and sperm count. Furthermore, the extract may have also influenced the action of mitochondria in the body of the tail of the spermatozoon, where the production of energy (in the form of adenosine triphosphate) for sperm motility takes place.

The administration of the *hydromethanol* extract of *Eugenia uniflora* in this present study did not significantly ($P<0.05$) the weights of the testes and epididymis. Although, there was a significant increase in testosterone and sperm count in extract administered groups, these changes did not cause alteration of testicular and epidydimal weights. Several studies indicate that reduced weights of testes and epididymis could result from low serum or plasma testosterone (Pokharkar *et al.*, 2010; Singh and Teotia (2017), The reports from another study suggest that low testicular or epidydimal weights could be as a result of disruption or hypoplasia of seminiferous tubules, erosion of germinal epithelium and disorganized histoarchitecture of the testes and disarray of sperm population dynamics (Priya *et al., 2*012). The findings in the present study indicate that an increase in testosterone may not cause significant increase in testicular and epidydimal weights which may be due to the period of administration of the extract.

CONCLUSION

The results of this study showed that extract of *Eugenia uniflora* improved the level of serum testosterone and the percentages of viable sperm cells, sperm cells with normal morphology and actively motile sperm cells as well as, sperm count thereby ameliorating lead induced reproductive toxicity. The result have shown that the extract improves sperm quality and quantity and protects the male reproductive function from the hazardous effect of environmental toxicants such as lead.

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