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Original Research Article

Evaluation of Testicular Antioxidative Potential and Anticholesterolaemic Effects of Leaf Extract of *Eugenia uniflora* (Pitanga Cherry) on Male Wistar Rats

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Abstract

Introduction: Several plants have been proven useful as antioxidant agents as a result of the abundance of bioactive substances embedded in the plant. *Eugenia uniflora* is a medicinal plant popular in the tropics where it is applied in traditional medicine practice for treatment of some illnesses but most of its acclaimed therapeutic effects have not been scientifically proven. ***Aim:*** The present study is aimed at assessing the testicular antioxidative and some biochemical effects of *Eugenia uniflora* in male Wistar rats. ***Methodology:*** A total of 30 male Wistar rats were divided into 5 groups of 6 animals each. Group I which served as the negative control received distilled water while group II (positive control) received 5 mg/kg Lead. Group III received 200 mg/kg BW of the extract and 5 mg/kg Lead, while Group IV received 400 mg/kg BW of the extract and 5 mg/kg Lead and the animals in Group V received 800 mg/kg BW of the extract and 5 mg/kg Lead. Administration was by oral gavage. At the end, the testes were harvested for analysis of testicular parameters. ***Result:*** The result showed a decrease in the level of Malondialdehyde (MDA) while Glutathione reductase (GSH) and Superoxide Dismutase (SOD) activities increased in some groups of animals treated with the extract and lead. The testicular total cholesterol level was significantly ($P<0.05$) decreased while the testicular protein level was not significantly affected in animals treated with the extract and lead when compared to the lead only group.

Keywords: Eugenia uniflora, Antioxidative, Wistar rats, Medicinal plant.

INTRODUCTION

Medicinal plants are used globally for treatment of various human ailments. Ethnomedicinal practice is still very much prevalent in developing countries (Maurga *et al.*, 2004). The World Health Organisation reported that about 80% of the entire world populations still rely on plant-based medicine for their health care (Osuagwu and Eme, 2013), Interestingly, medicinal plants has been proven to be effective in treatment of several diseases and disorders as well as made enormous contribution to the development of pharmaceuticals. It has been reported that, about 25% of modern drugs are derived from plants (Nantia *et al*., 2009). *Eugenia uniflora* L is a medicinal plant that belongs to the family Myrtaceae and is native to South America and mainly found in tropical and subtropical regions of the world (Kanazawa *et al*., 2000; Consolini & Sarubbio, 2002; Heywood *et al*., 2007; Wilson, 2011). It is a shrubby semi-deciduous tree with perennial leaves that are continuously available throughout the year (Kanazawa, Patin, & Greene, 2000). It is recognized and accepted in folk medicine as remedies for various ailments such as diarrhea and stomach problems, inflammation, rheumatism, hypertension and fever (Bakr, Mohammed & Waly, 2017). The biological effects of the plant have been attributed to the presence of several bioactive substances in the plant such as; tannins, flavonoids, triterpenpoids and alkaloids (Onwudiwe *et al*., 2010; Bakr, Mohammed &Waly, 2017). However, there are scanty scientific reports on the effects of *Eugenia uniflora* on testicular antioxidant status and some

testicular biochemical parameters. The present study is aimed at evaluating the effect of leaf extract of *Eugenia uniflora* on body weight, testicular oxidative stress and some testicular biochemical parameters in male wistar rats.

MATERIALS AND METHODS

Plant Material and Extraction

The *Eugenia uniflora* leaves used for this study were 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. Voucher specimen of the plant was deposited in the herbarium.The leaves were air dried for two weeks and ground into powder prior to its extraction at the Pharmacognosy department of the University of Port Harcourt, Nigeria. The process of extraction involved the dissolution of the powdery substance in a maceration jar containing 80% methanol and 20% water for 72 hours. The mixture was agitated three times daily for the three days it was allowed to stay. At the end of the maceration period, the substance was filtered with a white handkerchief and re-filtered with filter paper so as to get a clear filtrate. The filtrate was then concentrated using a rotary evaporator at an optimum temperature (40 – 50^0C). The filtrate containing the extract was poured into an evaporating dish and dried on a water bath at a temperature of 45^0C until it dried into a paste form.

Experimental Animals

A total of 30 male Wistar rats bred in the animal house of Department of Human Physiology, University of Port Harcourt was used as the experimental model. The animals which weighed between 65 – 100g at the beginning of the experiment were handled under laboratory conditions, in accordance to National and Institutional guidelines for animal being used for experimental purposes and fed with standard feeds and water. The animals were also allowed to acclimatize for 14 days before the experimental processes began.

Experimental Design

The male rats used for the study were divided into 5 groups of 6 animals each. Group I which served as the negative control received distilled water. Group II received 5 mg/kg of Lead, Group III received 200 mg/kg BW of the extract and 5 mg/kg of Lead, while Group IV received 400 mg/kg BW of the extract and 5 mg/kg of Lead and the animals in Group V received 800 mg/kg BW of the extract and 5 mg/kg of Lead. All the administration was by oral gavage once daily while the duration of the administration of the extract was 30 days to adult male Wistar rats. At the end of the administration period, the animals were weighed and sacrificed under light chloroform anesthesia and their testes harvested for analysis of testicular parameters.

STATISTICAL ANALYSIS

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

RESULTS

The results of this study are presented in tables 1-3.

Table-1: The effect of extract of E. uniflora on the Body weight of the Wistar rats

GROUPS	Initial Weight (g)	Final Weight (g)	Change in Body Weight (g)	% Difference in Weight
I	70.40 ± 3.49	155.80 ± 15.48	85.40	0
II	89.20 ± 8.77	172.00 ± 9.33	82.80	0
III	77.40 ± 7.60	190.60 ± 12.35	113.20$^{\#}$	36.71
IV	74.00 ± 6.79	170.20 ± 7.94	96.20	16.18
V	79.80 ± 4.18	165.40 ± 10.09	85.60	3.38

Values are expressed as mean ± SEM, n=6. $^{\#}$ Significantly (P<0.05) different from positive control group.

The initial body weight of the animals and their weight prior to sacrifice were determined and tabulated above as table 1. The table also highlights the difference between the two determined weights as well as the percentage difference across groups.

Table-2: The effect of extract of E. uniflora on testicular oxidative stress markers

GROUPS	GSH(µg) (µg/min/mg.protein)	CAT(µg) (Units/mg.protein)	SOD (µg) (Ug/mg.protein)	MDA (µg) (Umol/mg.protein)
I	1.36±0.04	0.63±0.13	0.18±0.03	0.64±0.04
II	1.67±0.07	0.77±0.08	0.20±0.04	65.00±3.73
III	3.74±1.99$^{*\#}$	0.80±0.03	0.24±0.03	60.80±2.70*
IV	1.99±0.07	0.83±0.05	0.31±0.06*	59.00±3.94*
V	2.53±0.12	0.96±0.13	0.48±0.05$^{*\#}$	36.40±6.23$^{*\#}$

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

The effect of the extract on oxidative stress parameters; Glutathione reductase (GSH), Catalase (CAT), Malondialdehyde (MDA), and Superoxide Dismutase (SOD) as analysed in this study is presented in Table 2 above. As seen in the table, the level of GSH, CAT and SOD enzyme activities in the various groups are recorded.

Table-3: The effect of extract of E. uniflora on Total testicular Protein and testicular Cholesterol.

GROUPS	Total Protein (mg/dl)	Total Cholesterol (mmol/l)
I	16.40±1.33	1.40±0.06
II	18.00±1.22	0.60±0.16*
VI	15.80±1.39	0.49±0.02*
VII	14.20±0.73	0.35±0.04*#
VIII	12.20±1.02	0.38±0.02*

Data are expressed as mean ± SEM, n=6. *# significantly (P<0.05) different from negative and positive control groups respectively

The levels of the testicular protein and testicular cholesterol analysed in this study as shown in Table 3 above. The mean level measured for each group is recorded against the group.

DISCUSSION

The present study describes the effects of oral administration of hydromethanol extract of *Eugenia uniflora* on body weight, testicular tissue cholesterol and protein levels and testicular antioxidant status in lead treated male Wistar rats. The administration of the hydromethanol extract of *E. uniflora* in the present study resulted in significant weight gain in the group that was treated with the lead and 200mg/kg of the extract. In a previous study, Sun *et al*. (2017), showed that lead water exposure caused weight gain in rats. However, Amjad, *et al*., (2013) reported a decrease in the body weight of rats exposed to lead. According to their findings, administration of lead resulted in noticeable reduction in body weight of the albino rats possibly caused by anorexia resulting from heavy metal ingestion or as resulting from decreased muscle mass and cachexia due to the oxidative stress induced by lead (Amjad, Iqbal &Shoro, 2013). However, the concentration of lead administered in this study did not cause significant changes in the body weight of rats but the lead administered in combination with low dose [200mg/kg] of extract caused marked increase in weight of the animals.This may suggest that the extract at lower doses [200mg/kg] may trigger certain physiological changes such as improved appetite or modulate satiety in the animals towards increased food intake.

Over the years, antioxidants have been identified to play significant roles in prevention of diseases including the development of degenerative diseases as a result of their ability to effectively stop the initiation and promotion of oxidative chain reactions which generate reactive oxygen species (Moure, Dominguez &Parajo, 2006). These agents are compounds or systems that function to delay autoxidation through inhibition of free radicals formation or interrupting free radical propagation by either of scavenging species that initiate peroxidation, chelating metal ions to inhibit their ability to generate reactive species, decompose lipid peroxides, preventing formation of peroxides and so on (Al-Attar, 2020). However, antioxidants of natural origin such as plant has been noted to have a great level of superiority over those of synthetic origin due to factors such as tolerance, safety and the absence of side effects (Mukhopadhyay, 2000). Patrick (2006) also demonstrated that lead toxicity may be mitigated by improving the cellular availability of antioxidant agents.

The present investigation showed that Lead (Pb) induced oxidative stress as indicated by changes in Glutathione (GSH), Catalase (CAT), Malondialdehyde (MDA), and Superoxide Dismutase (SOD) levels. This is because even small changes in oxidant or/and antioxidant levels, may disturb its balance and leads to oxidative stress (Bahrami *et al*., 2016). The use of lead in inducing oxidative stress has been postulated to be a major mechanism of lead associated tissue injury (Mohamed *et al*., 2016; Hou *et al*., 2019). In the present study, the MDA level was significantly increased in the lead treated groups to suggest an increase in lipid peroxidation. Malondialdehyde is a by-product of lipid peroxidation. Malondialdehyde production as well as generation of lipid peroxides, loss of membrane structure and function (Bas & Kalender, 2016; Okediran *et al*., 2017), while the increase in the GSH activity could be said to result from the increased presence of the metal in the body system. However, the administration of the extract decreased the MDA level, while increasing the GSH and SOD in lead treated rats. A similar finding to this shows that the application of *C. aconitifolius* as supplemented diet ameliorated MDA level which represents index of lipid peroxidation while increasing both GSH and CAT activities which also shows its ameliorative potentials against oxidative stress (Adaramoye & Aluko, 2010; Ngozi *et al*., 2018; Ajiboye *et al*. 2019). According to Rodrigues *et al*. (2005), the potential reduction of lipid peroxidation activity of an extract may be attributed to the presence

of saponins which have been found to exhibit antioxidant activity by reducing the lipid hydroperoxides level while the free radical scavenging activities due to the presence of flavonoids and vitamins C and E which possess antioxidant potentials have been implicated in the increase of some anti oxidative enzyme activities (Ong *et al*., 2011; Ajiboye *et al*., 2019; Us *et al*., 2020). Also, as stated by Ebuehi, Ogedegbe and Ebuehi (2012), though vitamin E is effective in the treatment of lead-induced toxicity, its combination with vitamins C produce synergistic and additive effects. Furthermore, the antioxidant activity shown by this extract may have been achieved through different mechanisms such as activation of the antioxidant enzyme systems and/or by free radical scavenging action and also could be attributed to the amount of phenolic compounds contained in it (Kulkarni *et al*., 2004).

Analysis of the testicular total protein in this study showed no significant difference between controls and test groups. The findings in this study also show that lead did not significantly affect total protein. The assay for tissue total protein level is to find out if there would be an indication of a disturbance of protein metabolism. The assessment of total protein is a rough measure of the protein status which can give rise to major changes in tissue functions while tissue toxicity arising from exposure to lead in any form may result in decreased levels of total protein (Kaneko *et al*., 2008). Nonetheless, the protein level was not significantly altered within the period of this study and the extract as well, did not significantly alter protein level.

In respect of the tissue total cholesterol, exposure to lead caused significant ($p<0.05$) reductions in tissue total cholesterol level. Furthermore, the extract further decreased total cholesterol level in the group that received 400mg/kg of extract when compared to the positive control (Lead group). The tissue cholesterol play an important role in the synthesis of steroid hormones. Thus, the further decrease in testicular cholesterol level may indicate increased mobilization as occurs in the synthesis of steroid hormone, thereby making it imperative for the study of the effects of Eugenia uniflora on steroid hormones such as the sex hormones.

CONCLUSION

It could be deduced from the result of the present study that the administration of the leaf extracts of *E. uniflora* could modulate changes in body weight and also play a huge role in protecting the rat against harmful and dangerous effects of oxidative stress.

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