wjpmr, 2023, 9(2), 186-190

SJIF Impact Factor: 6.842

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

NEPHROTOXICITY ATTENUATION BY *PERSEA AMERICANA'S* ETHANOLIC SEED EXTRACT IN EXPERIMENTAL WISTAR RATS MODEL OF ETHYLENE GLYCOL-INDUCTION

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Article Received on 21/12/2023 **Article Revised on 11/01/2023** **Article Accepted on 01/02/2023**

ABSTRACT

Five (5) classes with five (5) rodents each were in experimental design to study impact of *Persea americana's* seed extract on induced-nephrotoxicity. First group receiving animal feed and water *ad libitum* served for control, Group 2, positive control receiving 0.1ml/kg b.w of ethylene glycol for 7 days, Groups 3 and 4 served as treatment groups receiving 100mg/kg and 200mg/kg extract of *Persea americana* (EPA), twice daily for 21 days and 0.1ml/kg ethylene glycol (EG) for 7 days with continuation of the treatment of EPA and Group 5 received a reference drug of 0.2mg/kg Folic acid and 0.3mg/kg Thiamine (twice daily) for 21 days and 0.1ml/kg EG for 7 days with continues treatment of the reference drug. Nephroprotective effects of EPA was evaluated by estimating the activities of Urea, Creatinine and levels of total protein. The effects of EPA on biomarkers of oxidative damage (lipid peroxidation) and antioxidant enzymes namely, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were also measured in the kidney using homogenates of the organ. EPA and the reference drug used revealed nephroprotective action for reducing ($p< 0.05$) Urea with creatinine when compared to the positive control. Measures of oxidative stress bio-indicators were depleted along graded dose in EPA – administered animals. Conclusively, EPA may have proffered nephroprotection not unconnected with its antioxidative bioactivity.

KEYWORDS: Persea Americana, ethylene glycol, nephrotoxicity, nephroprotective, antioxidants

INTRODUCTION

Ethylene glycol (EG) is a toxic substance that is commonly used as an antifreeze in automobiles, but it can also be found in other household products. It is also known by its chemical formula, $C_2H_6O_2$, and its systematic name, ethane-1,2-diol (Khetan and Collins, 2007; Hwang *et al.*, 2017). When EG metabolize, it's converted into toxic compounds that can cause damage to the kidneys, nervous system, and other organs.

Avocado is one of the plants that have been widely used in ethno-medicine. In Nigeria, the fruit has various local names such as Ewé pia (Yoruba), Ube oyibo (Igbo), and Ganyen piya (Hausa). Avocado fruits have culinary and nutritional values. Its seeds are rich in bioactive phytoconstituents (Lara-Marquez *et al.*, 2020; Saavedra *et al.*, 2017; Dabas*et al.*, 2019; Uchenna*et al.*,2017; Soledad *et al*, 2021; Villarreal-Lara *et al.*, 2019),

Meanwhile, EPA has not so much been reported with regards to nephroprotection, thus ethanolic derivation of it was examined in wistar rats exposed to ethylene glycol – triggered kidney damage.

METHODOLOGY

Avocado pears were purchased freshly and the flesh striped revealing the endocarp (seed). These seeds were then washed, dried and crushed into powder. Approximately 400grams of the crushed powder was weighed and homogenized with one (1) litre of 90% ethanol in glass jars and allowed to stand for 48 hours with intermittent stirring. The mixture was then filtered and residue was placed inside a rotary evaporator and it extracts collected, stored in sample bottles and refrigerated until time of use.

Experimental Animals

Twenty-five male Wistar strain rats purchased at Niger Delta University and acclimatized for 2 weeks under standard conditions of laboratory specification.

Experimental Design

Group 1 (normal control): received animal feed and distilled water *ad libitum.*

Group 2 (positive control) received ethylene glycol (0.1ml/kg b/w) for seven days.

Group 3 treated with ethanolic extracts of avocado pear seed at a dose of [100mg/kg b.w/twice daily]

Group 4treated with ethanolic extracts of avocado pear seed at a dose of [200mg/kg b.w/ twice daily].

Group 5 treated with a reference drug of 0.2mg/kg b.w folic acid and 0.3mg/kg b.w thiamine (both twice daily)

Twice daily is at morning and evening, oral administration in 21 days.

All rodents except group 2 were from day 22 given 0.1ml/kg b.w ethylene glycol for seven days with continuation of the treatment doses of EPA. After 28 days, wistar rats are euthanized, and blood aspirated into containers for the various biochemical analysis. The kidneys were harvested and part of it was homogenized using normal buffer and stored in labeled containers for the antioxidant analysis.

KIDNEY ASSAY

Total protein in the serum was measured using spectrophotometry in line with instructions in the Randox biochemical kit's manual; also for urea and creatinine.

Antioxidant assays

Tissue was cut, rinsed with cool tissue preservative, and following standard protocol A 10% (w/v) homogenate was prepared (Beutler, 1989), centrifugation was at 1000 x g for 10 minutes to remove cell debris and nuclei. The supernatants were kept at -80°C for the determination antioxidant activities. All centrifugations were carried out at 4°C using the refrigerated centrifuge. Antioxidant analysis was by the protocols of (Misra, & Fridovich 1972; Beutler, 1989; Paglia & Valentine, 1962).

Histopathological examination

After sacrifice and dissection, the kidneys from each rat were rapidly excised and then perfused in saline solution. Pieces from the organs of each rat (5mm3) were taken and fixed in 10% neutral buffered formal-saline. The fixed organs was sent to Histology Department, Bayelsa Medical University where further processing was done by the protocol of (Mehranjani *et al.*, 2009).

Statistical analysis

Analysis of all observation was accomplished by ANOVA and LSD statistical tools, with results in tables.

RESULTS

Biomarkers of kidney function

The effects of treatment with ethanolic extracts of avocado pear seed on serum Urea, Creatinine with total protein levels for ethylene glycol administered intoxicated wistar strain presents below.

Table 1: Mean Effect of Ethanol Extract of Avocado Pear Seed on Kidney Function Biomarkers (Urea, Creatine, Total Protein) of Wistar Rats.

Groups/Parameters	UREA (mg/dl)	Creatinine (mg/dl)	Total Protein (g/dl)
Group 1(Normal control)	50.97 ± 1.23^{b}	0.66 ± 0.02^{b}	9.21 ± 0.40^{b}
Group 2 (Positive control)	117.65 ± 1.88^{a}	1.94 ± 0.06^{a}	4.21 ± 0.20^{a}
Group 3 (100mg/kg b/w extract (twice daily) + EG)	84.44 ± 1.25^{ab}	1.07 ± 0.02^{ab}	6.10 ± 0.14^{ab}
Group 4 (200mg/kg b/w extract (twice daily) + EG)	75.94 ± 1.37^{ab}	0.97 ± 0.06^{ab}	7.86 ± 0.12^{ab}
Group 5 (Reference drug + EG)	78.42 ± 1.54^{ab}	0.99 ± 0.05^{ab}	6.25 ± 0.29^{ab}

Values are given as Mean ± SEM where SEM is the Standard Error of Mean. Different Superscript indicate significance ($p<0.05$) in comparison.

Total protein reduced ($p<0.05$), in ethylene glycol induced animals, while extract of avocado pear seed (twice daily) resulted in substantial increase. The reference drug decreased ($p<0.05$) urea, creatinine activity relative to the positive control group but elevated activity for Total protein than the positive control.

Biomarkers of oxidative damage

As seen below; Treatment with EG elevated antioxidant activities in (Group 2) than the normal (group 1) while, administration with EPA, the reference drug significantly ($p < 0.05$) increased the antioxidant activity as compared to the positive control (group 2).

Table 2: Mean Effect of Ethanol Extract of Avocado Pear Seed on Antioxidant and Oxidative Stress (SOD, CAT, GPx and MDA) on the Kidney of Wistar Rats.

Groups/Parameters	SOD (U/mg)	CAT(U/mg)	GPx(U/mg)	MDA(U/mg)
Group 1(Normal control)	7.95 ± 0.21^{b}	5.43 ± 0.16^{b}	8.36 ± 0.48^{b}	1.77 ± 0.10^{b}
Group2 (Positive control)	3.12 ± 0.10^{a}	1.69 ± 0.14^{a}	2.82 ± 0.14^{a}	5.64 ± 0.14^{a}
Group 3 (100mg/kg b/w extract (twice daily) + EG)	5.96 ± 0.12^{ab}	4.06 ± 0.07^{ab}	5.43 ± 0.14^{ab}	3.08 ± 0.12^{ab}
Group 4 (200mg/kg b/w extract (twice daily) + EG)	6.49 ± 0.24^{ab}	4.71 ± 0.22^{b}	5.72 ± 0.33^{ab}	4.01 ± 0.05^{ab}
Group 5 (Reference drug + EG)	5.53 ± 0.10^{ab}	4.50 ± 0.35^{ab}	6.39 ± 0.09^{ab}	3.94 ± 0.08^{ab}

Values with different Superscript indicate significant; (p<0.05) comparing with Group 1, and 2 respectively.

Impact of EPA administration; it reversed lipid peroxidation activity in EG –induced rats, where EG caused significant elevation in MDA levels relative to control. Similarly, the reference drug used also significantly decreased these by-products when compared to the positive control group.

Histopathology
Kidney

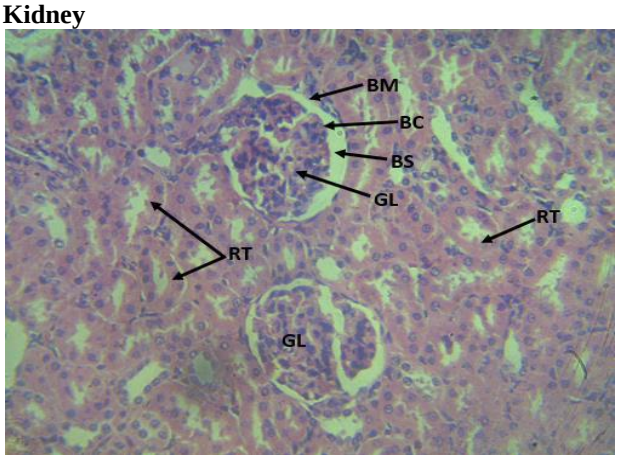

Plate 1: Normal Control (Group 1).

Photomicrograph (H&E X400) of the Kidney showing a normal structure of the renal corpuscles with the Glomeruli (GL): visible Bowman's capsule (BC), bowman's space (BS); also the basement membrane (BM) and renal tubules (RT).
Diagnostics: Normal Kidney tissue.

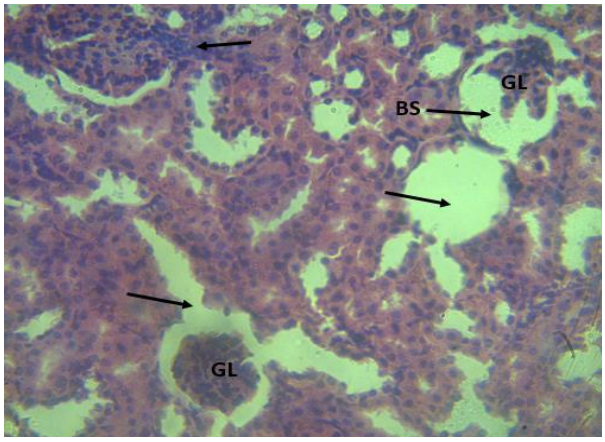

Plate 2: Positive Control (Group 2) 0.1ml/kg b/w Ethylene glycol.

Photomicrograph (H&E X400) of the Kidney showing interstitial oedema, mononuclear infiltration (arrows) and glomerular necrosis associated with dilatation of the bowmans space and glomerular shrinkage.

Diagnosis: Degeneration of the kidney tissue.

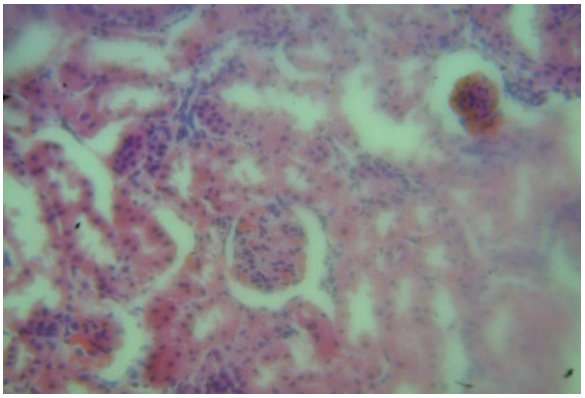

Plate 3: 100mg/kg b/w (twice daily) Group 3.

Photomicrograph (H&E x400) of the kidney with moderate inflammatory cells infiltration of the renal tubules with associated glomerular atrophy and dilatation of bowman's space (arrows).

Diagnostic: Moderate renal tubular degeneration.

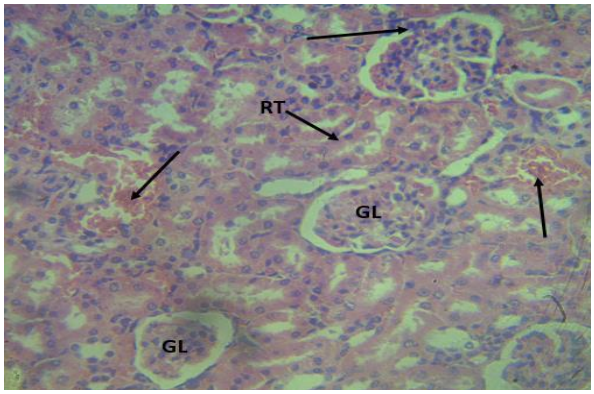

Plate 4: 200mg/kg b/w (twice daily) Group 4.

Photomicrograph (H&E X400) of the Kidney with mild mononuclear infiltration of the glomerulus (GL), renal tubules (arrows).

Diagnostics: Mild inflammation of the glomerulus

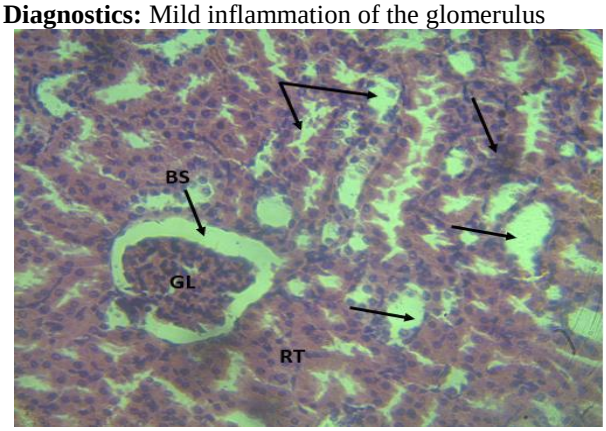

Plate 5: 0.6mg/kg thiamine and 0.4 mg/kg Folic acid Group 5.

Photomicrograph (H&E X400) of the Kidney with diffused interstitial oedema, mononuclear infiltration of

the renal tubules (RT); with mild dilatation of bowman's space.

Diagnostics: Mild renal tubules inflammation and vacoulation.

DISCUSSION

EPA was investigated if it might ameliorate kidney damage in wistar rats intoxicated with EG. Results reveal that EG -induction caused Urea, creatinine elevation - depicting nephrotoxicity and total protein level depletion – indicating oxidative stress, but pretreatment by EPA reversed the bio –indicators in those animals; in tandem with study by (Burtis *et al*, 2008; Ezejiofor *et al*, 2013). Ejiofor and his research team assert that avogado fruit and seed acts as nephro – protective agent which may in - turn improve deteriorated kidney function parameters.

In the same vein, histopathological analysis showing tubular degeneration, inflammation and vacoulation further confirms extent of nephrotoxicity. EG metabolism into toxic metabolites including glycoaldehyde, glycolic acid, glyoxylic acid, and oxalic acid harms the kidney; and adverse renal effects after EG ingestion typically occur after acute exposure (Hess *et al.*, 2004). But these were ameliorated or reversed by treatment with EPA.

Furthermore, increased lipid peroxidation was implicated in EG – administered rats by MDA levels. Meanwhile, treatment with EPA decreased MDA concentrations, implying nephroprotection; most probably connected with its composition by bioactive agents (Gupta *et al.*, 2004; Mankani *et al.*, 2005; Arukwe *et al.*, 2012).

Moreover, CAT, GPx, SOD is heightened by EG, implicating oxidation stress (John *et al.*, 2001), which was however decreased in EPA administered animals. It thus appears perhaps, that EG triggered free radicals and harmed the kidney, whereas EPA by virtue of its phytoconstituents' bioactivity mopped free radicals and relieved the kidney.

CONCLUSION

Extract of *Persea americana* has nephroprotective health benefit as demonstrated in experimental model of ethylene glycol – triggered nephrotocity; by mechanisms which may be associated with its antioxidant phytoconstituents.

ACKNOWLEDGEMENT

The authors wish to acknowledge that this particular research was funded by TETFund (Tertiary Education Trust Fund), Nigeria.

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