Original Research Article

Antioxidant potentials of *Pentaclethra macrophylla* seed (Ugba) on Mercury toxicity induced hepatic, renal and testicular oxidative stress in male Albino Rats

.

ABSTRACT

Aim: The aim of this study was to antioxidant potentials of *Pentaclethra macrophylla* seed (Ugba) on mercury toxicity induced hepatic, renal and testicular oxidative stress in male Albino Rats.

Study design: This study is an experimental study.

Place and Duration of Study: Department of Animal and Environmental Biology Animal House, Rivers State University, Port Harcourt, Nigeria, between January, 2019 and January, 2021.

Methodology: Thirty six (36) adult male albino rats weighing approximately 135±1.5g were purchased from University of Port Harcourt, Rivers State, Nigeria. They were housed in plastic suspended cages, placed in well ventilated conditions and provided with rat diet and water and cclamatize for two weeks. Fresh matured seeds of *Pentaclethra macrophylla* (African oil bean seed) were sourced locally from markets in Imo state, Nigeria. The washed and sliced seeds were stored at room temperature and allowed to ferment. The fermented seeds were dried, ground and preserved in airtight container in the refrigerator at 4^{0c}.The total weight of the powdered seed of *Pentaclethra macrophylla* produced was 250g Seeds were thoroughly washed with distilled water, cooked for more than 2hours at $100^{0c.}$ Maceration technique was used to carry out an ethanolic extract of the plant seed. Mercury chloride salt was purchased in Port Harcourt. A standard dose of 3.0mg/kg body weight of mercury chloride obtained from acute toxicity study dose determination was administered to the rats for 30 days after they were divided into six groups of six rats per group. After 30 days, all the animals were weighed, anaesthetized using chloroform. Tissue samples of liver, kidney and testis were collected and homogenized in phosphate buffered saline and stored at 4^0C for determination of oxidative stress assessment. Malondialdehyde, Reduced Glutathione (GSH), Catalase Activity, superoxide dismutase (SOD) activity, Glutathione Peroxidase Activity were analysed using the homogenized sample. Data were expressed as mean ±SD, and the statistical analysis was performed with the SPSS statistics 23.0 and p values less than .05 were considered statistically significant.

Results: The results showed that there were significant increases (*P<.05*) in Liver, kidney and testicular MDA and significant decreases (*p<.05)* in SOD, CAT, GSH and GPx activities in group 2 rats versus control. However, after treatment with different concentrations of *Pentaclethra macrophylla,* MDA activity significantly decreased (*P<.05*), while SOD, CAT, GPx and GSH significantly increased (*P<.05*).

Conclusion: The results obtained from the study showed that mercury chloride has the potential to cause liver, kidney and testicular toxicity to adult male albino rats and this toxicity was exerted majorly through oxidative stress and lipid peroxidation. However, treatment with *Pentaclethra macrophylla* seed extract ameliorated the oxidative stress.

Keywords: Antioxidant, Pentaclethra macrophylla seed (Ugba), Mercury toxicity, male Albino Rats

1. INTRODUCTION

Mercury is a well - known industrial, multi – organ, and environmental toxicant, which induces harsh alterations in organs and tissues of both humans and animals [1]. As a result of its ever – present nature, mercury exposure occurs through diverse forms, such as, the eating of infected fish, breathing of mercury vapour or industrial exposure. Mercury exits in different forms, like, metallic, organic and inorganic mercury [1, 2]. The entire forms of mercury potentiate toxicity in numerous organs and tissues, depending on factors like, level of exposure, duration of exposure, portal of exposure and chemical form of mercury [1]. It is nearly unfeasible for humans to invade exposure to various forms of mercury as a result of its ever – present nature caused by environmental changes and industrialization. Human deaths occasioned by professional exposure of mercury chloride had earlier been recognized and reported [3]. Mercuric chloride salt is weakly soluble in water and weakly absorbed by the intestine, though few section is thought to go through oxidation to absorbable forms [2]. On entry to the blood circulation, metallic mercury is oxidized to mercuric mercury from where it could be elated and deposited in the kidneys, testes and liver, which may be connected with the malfunction of these organs. Additionally, mercury intoxication disorganizes intracellular thiol content in a way that provokes oxidative stress, perturbation in heme metabolism, mitochondrial malfunction and lipid oxidation [4]. Oxidative stress driven histological alteration in kidney tissues exposed to mercury has been documented. Elevated lipid oxidation and serum activities of gamma – GT, AST and ALT, suggestive of liver damage have been documented in investigational mercury intoxication. Despite considerable progress in understanding the underlying mechanisms of mercury toxicity and available treatment or management option, mercury intoxication is still a serious health concern [5]. Due to their ubiquitous nature, humans are at high risk of exposure to mercury, their metabolites and derivatives, which are capable of disrupting hormonal, renal and hepatic functions; [5, 6]. The use of plants and natural products in the therapeutic management of testis, liver, kidney and other tissue associated toxicity possibly due to fewer side effects is a focus of most research models. Thus, the search for viable therapeutic candidates of plant origin that could protect against mercury–induced organ damage is desirable. Therefore, the aim of this study was to antioxidant potentials of *Pentaclethra macrophylla* seed (Ugba) on mercury toxicity induced hepatic, renal and testicular oxidative stress in male Albino Rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty - six (36) adult male albino rats weighing approximately 135±1.5g were purchased from University of Port Harcourt, Rivers State, Nigeria and were used for this research work. The animals were housed in plastic suspended cages, placed in well ventilated conditions and provided with rat diet and water. The ethical regulations on animal care and handling of the National Academy of Science were observed and the rats were made to acclimatize for 14 days prior to experiment.

2.2 Collection and identification of Plant Materials

Fresh matured seeds of *Pentaclethra macrophylla* (African oil bean seed) were sourced locally from markets in Imo state, Nigeria. The seeds were identified by Dr. Mbagwu V. of the department of plant science and Biotechnology, Imo state University, Owerri.

2.3 Preparation of *Pentaclethra macrophylla* powder from Seed

Seeds were thoroughly washed with distilled water, cooked for 2hours at 100^{0c} to remove the seed coats, washed three times, sliced and cooked for another 2hours at 100^{0}C, rinsed three times with distilled water [7]. The washed and sliced seeds were stored at room temperature and allowed to ferment. The fermented seeds were dried, ground and

UNDER PEER REVIEW

preserved in airtight container in the refrigerator at 4^{0c}.The total weight of the powdered seed of *Pentaclethra macrophylla* produced was 250g.

2.4 Ethanol Extraction (Maceration method) [8]

Maceration technique was used to extract the phytochemical content of the plant seed used in this research work. 52g of the ground seed powder was soaked in 100mls of ethanol and allowed to stand at room temperature of 25^0C for 72 hours with agitation at interval of one hour. At the end of the third day, the content was filtered using Whatman no 1 filter paper and filtrate was subjected to rotary evaporator to remove the ethanol content. The recovered solute (powder) was then weighed again to enable the calculation of percentage yield.

2.5 Calculation for Percentage yield of Ethanol Seed Extract

To calculate the percentage yield of ethanol seed extract (g) = W1 x 100/ W2

Where,

W1 = Weight of dried powder of the extract obtained after solvent removal = 52grams.

W2 = Weight of the dried powdered seed sample before adding to solvent = 100grams.

To calculate the percentage yield of ethanolic seed extract = $\frac{52 \times 100}{100} = 52\%$

2.6 Preparation of Seed extract for Treatment

Following the ethanolic extraction and evaporation of ethanol, a total of 52grams of the seed in powdered form were recovered. 52grams of the post – ethanolic extract powder was dissolved in 100mls of corn oil. Therefore, 1.0ml of the solution contain 0.5g/ml of the extract. Therefore, in 135±1.5g rats, 0.5g/ml will be equivalent to 3.7g/kg body weight of rats.

2.7 Procurement and Preparation of Mercury Chloride Salt

Mercury chloride salt was purchased in Port Harcourt from Joe Kings Chemicals and made in China in a granular form. The salt is of industrial grade of 99.5% purity. Since the salt was purchased in a granular form, there was a need to dissolve the salt in Corn oil to facilitate oral treatment in the rats. 3.0mg of mercury chloride were weighed and dissolved in sterile container containing 8.0ml of corn oil. The contents of the container were mixed to ensure complete dissolution of the salt. This implies that 1.0ml of this solution contains 0.375mg of mercury chloride.

2.8 Administration of Mercury Chloride and *Pentaclethra macrophylla* seed extract

The method of treatment in the acute studies involved oral technique. In the oral treatment, mercury chloride salt and *Pentaclethra macropohylla* seed extract were administered using gavage tube inserted directly into the oesophagus of the rats through the mouth to ensure complete delivery of the salt and seed extract respectively.

2.9 Reagents and Chemicals

Mercury chloride (Kermel, China), tetraoxosulphate vi acid, sulphanilamide, hydrochloric acid, thiobarbituric acid, trichloroacetic acid, sodium hydroxide, ethylene - diaminetetraacetic acid ,sodium pyrophosphate, 5, 5 – dithiobis – 2 – nitrobenzoate, sodium nitroprusside, urease, phenol, picric acid, 2, 4 – dinitrophenyl hydrazine (UK), were obtained in Portharcourt.

2.10 Toxicity Induction with Mercury Chloride (HgCL2)

After two weeks of acclimatization, toxicity was induced in the male albino rats with mercury chloride ($HgCL_2$). A standard dose of 3.0mg/kg body weight of mercury chloride obtained from acute toxicity study dose determination was used to induce organ toxicity in the

UNDER PEER REVIEW

experimental rats. Organ and Tissue damage were determined by evaluating liver, kidney and testicular parameters under review.

2.11 Experimental Design

Thirty six (36) male adult albino rats weighing approximately 135±1.5g/kg were used for this research study. The animals were placed into six groups, each containing six rats and the duration of the experiment was 30 days. Mercury (II) chloride ($HgCl_2$) was dissolved in corn oil and administered three times a week by oral gavage. The administered dose of 3.0mg/kg body weight was done orally using gavage tube. *Pentaclethra macrophylla* seed extract was dissolved in corn oil and administered to the rats daily for 30 days.

Group 1: Control (rats received corn oil at 2ml/kg)
Group 2: $HgCl_2$ (rats were administered 3.0 mg/kg $HgCl_2$)
Group 3: 100mg/kg P.M + $HgCl_2$ (rats were treated with 100mg/kg P. M. and 3.0mg/kg $HgCl_2$)
Group 4: 200mg/kg P.M + $HgCl_2$ (rats were treated with 200mg/kg P. M. and 3.0mg/kg $HgCl_2$)
Group 5: 100mg/kg P.M (rats were treated with 100mg/kg *Pentaclethra macrophylla* alone)
Group 6: 200mg/kg P.M (rats were treated with 200mg/kg *Pentaclethra macrophylla* alone)

2.12 Blood and Tissue samples Collection and Preparation.

After 30 days, all the animals were weighed, anaesthetized using chloroform. Tissue samples of liver, kidney and testis collected were homogenized in phosphate buffered saline and stored at 4^0C for determination of oxidative stress assessment.

2.12.1 Homogenization.

The liver, kidney, and testicular tissues were rinsed with ice cold KCl solution, and blotted with filter paper and weighed. They were then chopped into bits and homogenized in nine volumes of the homogenizing buffer (0.1M Phosphate buffer Saline, pH 7.4). The homogenizing buffer was prepared by dissolving 0.496 g of di-potassium hydrogen orthophosphate, K_2HPO_4 and 0.973 g of potassium di-hydrogen orthophosphate, KH_2PO_4 in 9 ml of distilled water. The pH was adjusted to 7.4 and then made up to a 100 ml with distilled water. The resulting homogenate was centrifuged at 10,000 rpm for 10 minutes in a cold centrifuge (4^oC), to obtain the post mitochondrial fraction. The supernatant was collected and used for oxidative stress parameters analyses.

2.13 Laboratory Procedures.

2.13.1 Estimation of Biomarkers of Oxidative Stress.

2.13.1.1 Determination of Malondialdehyde by Ohkawa et al. [9].

Principle

Malondialdehyde (MDA) a marker of lipid peroxidation was assessed as thiobarbituric acid reactive substances (TBARS) in the sample. In acidic medium, thiobarbituric acid (TBA) interacts with MDA generated from peroxidation of fatty acids to yield a pink coloured solution

2.13.1.2 Determination of Reduced Glutathione (GSH) by Moron et al. [10]

Principle

The reduced glutathione exists largely as non-sulfhydryl groups in samples and it interacts with Ellman's reagent (5′, 5′–dithiobis-(2-nitrobenzoic acid) (DTNB) to generate a stable yellow coloured chromogenic solution with molar absorption at 412nm.

UNDER PEER REVIEW

2.13.1.3 Determination of Catalase Activity

Principle
The method is based on the loss of absorbance observed at 240 nm as catalase splits hydrogen peroxide. Despite the fact that hydrogen peroxide has no absorbance maximum at this wavelength, its absorbance correlates well enough with concentration to allow its use for a quantitative assay. An extinction coefficient of 0.0436 $mM^{-1}cm^{-1}$ was used.

2.13.1.4 Determination of superoxide dismutase (SOD) activity by Misra and Fridvich, [11]

Principle
The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH10.2 makes this reaction a basis for a simple assay for this dismutase. Superoxide (O2•-) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O2•- introduced increased with increasing pH and also increased with increasing concentration of epinephrine. These results led to the proposal that autoxidation of epinephrine proceeds by at least two distinct pathways, only one of which is a free radical chain reaction involving superoxide (O2•-) radical and hence inhibit-able by superoxide dismutase.

2.13.1.5 Determination of Glutathione Peroxidase Activity (GPx) by Paglia and Valentine, [12]

Principle.
The assay is an indirect measure of the activity of c-GPx oxidized glutathione (GSSG) produced upon reduction of an organic peroxide by c GPx, is recycled to its reduced state by the enzyme, glutathione reductase (GR).

$$R - 0 - 0\,H + 2GSH \xrightarrow{c - GPx.} R - 0 - H + GSSG + H20.$$

$$GSSG + NADPH + H^{+} \xrightarrow{GR} 2GSH + NADP^{+.}$$

The oxidation of NADPH to $NADP^{+}$ is accompanied by a decrease in absorbance at 340nm wavelength providing a spectrophotometric means for monitoring GPx enzyme activity. The molar extinction coefficient for NADPH is 6220 m^{-1} cm^{-1} at 340nm. The assay c- GPx, a cell or tissue homogenate is added to a solution containing glutathione, glutathione reductase and NADPH. The enzyme reaction is initiated by the addition of the substrate and hydrogen peroxide. The rate of decrease in the absorbance at 340nm is directly proportional to the GPx activity in the sample [12].

2.14 Statistical Analyses

Data were expressed as mean ±SD, and the statistical analysis was performed with the SPSS statistics 23.0 (SPSS Inc. Chicago, IL). All the statistical analysis were analysed by ANOVA followed by Tukey's multiple test. *P<.05* were considered to indicate statistical significance.

UNDER PEER REVIEW

3. RESULTS AND DISCUSSION

Table 1: Liver MDA. SOD and GSH concentrations of rats exposed to mercury chloride ($HgCl_2$) and *Pentaclethra macrophylla* (PM) Seed Extract for thirty (30) days

Parameters/Grps	MDA (µmol/mg protein)	SOD (U/mg protein)	GSH (U/mg protein)	P-value	Remark
1. Control	9.48±1.63	63.45± 5.07	1.72±0.65	0.217	NS
2. HgCl (3.0mg/kg)	47.45±7.91[a]	24.74 ± 1.68[a]	0.60± 0.51[a]	0.000	S
3.HgCl (3.0mg/kg)+PM(100mg/kg)	28.91±6.99[b]	68.43± 9.79[b]	1.68± 0.36[b]	0.000	S
4.HgCl (3.0mg/kg)+PM(200mg/kg)	16.71±3.82[a]	81.04±12.37[a,b]	3.01±0.52[a,b]	0.007	S
5.PM (100mg/kg)	10.12±1.74[b]	52.11± 3.52[b]	2.26 ± 1.14[b]	0.112	NS
6.PM (200mg/kg)	6.33±1.62[b]	59.97±5.24[a,b]	2.58±0.59[a,b]	0.000	S

Each value represents the mean ± SD; [a]Significantly different from the control; [b]Significantly different from $HgCl_2$ ($p < 0.05$).
Key: MDA – Malondialdehyde
S - Significant
NS - Not Significant.
SOD – Superoxide Dismutase
GSH – Reduced glutathione

Table 2: Liver CAT and GPx activities of rats exposed to mercury chloride ($HgCl_2$) and *Pentaclethra macrophylla* (PM) Seed Extract for thirty (30) days

Parameters/Grps	CAT (U/mg protein)	GPx (U/mg proteins)	P-Value	Remark
1. Control	71.36±19.18	1.02±0.18	0.276	NS
2. HgCl (3.0mg/kg)	16.96±4.58[a]	0.07±0.29	0.002	S
3.HgCl(3.0mg/kg)+PM(100mg/kg)	52.69±19.79[b]	1.09±0.15	0.002	S
4.HgCl(3.0mg/kg)+PM(200mg/kg)	63.78±23.44[b]	1.47±0.51	0.003	S
5.PM (100mg/kg)	59.85±14.72[b]	1.56±0.49	0.001	NS
6.PM (200mg/kg)	93.99±9.17[a,b]	1.93±0.45	0.000	S

Each value represents the mean ± SD; [a]Significantly different from the control; [b]Significantly different from $HgCl_2$ ($p < 0.05$).
Key: CAT – Catalase
S - Significant
NS - Not Significant.
GPx – Glutathione peroxidase

Table 3: Kidney MDA, GSH and SOD concentrations of rats exposed to mercury chloride ($HgCl_2$) and *Pentaclethra macrophylla* (PM) Seed Extract for thirty (30) days

Parameters/Grps	MDA (µmol/mg protein)	GSH (U/mg protein)	SOD (U/mg protein)	P-value	Remark
1. Control	7.79 ± 1.07	1.53±1.55	57.58 ± 7.32	0.256	NS
2. HgCl (3.0mg/kg)	36.34 ± 5.35[a]	0.85± 0.19	22.06 ± 2.90[a]	0.000	S
3.HgCl(3.0mg/kg)+PM(100mg/kg)	15.77 ± 1.55[a,b]	2.07±0.6	54.8 ± 6.21[b]	0.000	S
4.HgCl(3.0mg/kg)+PM(200mg/kg)	8.77±0.85[b]	2.13±1.07	65.51 ± 7.21[b]	0.002	S
5.PM (100mg/kg)	8.14±1.13[b]	1.89±0.74[a,b]	48.2 ± 5.83[a,b]	0.113	NS
6.PM (200mg/kg)	3.52 ±0.34[a,b]	2.68±0.75[a,b]	114.67±12.59[a,b]	0.001	S

Each value represents the mean ± SD; [a]Significantly different from the control; [b]Significantly different from $HgCl_2$ ($p < 0.05$).

UNDER PEER REVIEW

Key: MDA - Malondialdehyde
S - Significant
NS - Not Significant.
GSH - Reduced Glutathione
SOD - Superoxide Dismutase

Table 4: Kidney CAT and GPx activities of rats exposed to mercury chloride ($HgCl_2$) and *Pentaclethra macrophylla* (PM) Seed Extract for thirty (30) days

Parameters/Grps	CAT (U/mg protein)	GPx (U/mg protein)	P-Value	Remark
1. Control	55.07±13.39	1.09±0.28	0.022	S
2. HgCl (3.0mg/kg)	12.85±3.30[a]	0.61±0.47	0.001	S
3.HgCl(3.0mg/kg)+PM(100mg/kg)	35.50±5.85[a,b]	0.89 ±0.12	0.002	S
4.HgCl(3.0mg/kg)+PM(200mg/kg)	43.19±6.80[b]	1.21±0.14	0.003	S
5.PM (100mg/kg)	47.31±8.09[a,b]	1.01 ±0.11	0.013	S
6.PM (200mg/kg)	71.02±8.43[a,b]	1.35±0.18[a,b]	0.000	S

Each value represents the mean ± SD; [a]Significantly different from the control; [b]Significantly different from $HgCl_2$ ($p < 0.05$).
Key: CAT - Catalase
S - Significant.
NS - Not Significant
GPx - Glutathione peroxidase

Table 5: Testis MDA, SOD and GSH concentrations of rats exposed to mercury chloride ($HgCl_2$) and *Pentaclethra macrophylla* (PM) Seed Extract for thirty (30) days

Parameters/Grps	MDA (µmol/mg protein)	SOD (U/mg protein)	GSH (U/mg protein)	P-value	Remark
1. Control	6.83±0.55	53.43±6.38	1.61±0.67	0.279	NS
2. HgCl (3.0mg/kg)	32.51±2.55[a]	20.24±2.46[a]	0.66±0.52a	0.000	S
3.HgCl(3.0mg/kg)+PM(100mg/kg)	11.34±3.25[b]	50.21±3.07[b]	2.23±0.94[b]	0.000	S
4.HgCl(3.0mg/kg)+PM(200mg/kg)	6.39±1.86[b]	59.07±4.62[b]	3.61±0.53[a,b]	0.002	S
5.PM (100mg/kg)	7.25±0.57[b]	42.41±5.20[a,b]	2.11±0.39[a]	0.230	NS
6.PM (200mg/kg)	2.50±0.72[a,b]	105.66±6.49[a,b]	3.25±0.62[b]	0.000	S

Each value represents the mean ± SD; [a]Significantly different from the control; [b]Significantly different from $HgCl_2$ ($p < 0.05$).
Key: MDA - Malondialdehyde
S - Significant
NS - Not Significant.
SOD - Superoxide Dismutase
GSH - Reduced glutathione

Table 6: Testis CAT and GPx activities of rats exposed to mercury chloride ($HgCl_2$) and *Pentaclethra macrophylla* (PM) Seed Extract for thirty (30) days.

Parameters/Grps	CAT (U/mg protein)	GPx (U/mg proteins)	P-Value	Remark
1. Control	60.77 ±11.91	1.19±0.15	0.005	S
2. HgCl (3.0mg/kg)	14.26 ±3.02[a]	0.70±0.27	0.001	S
3.HgCl(3.0mg/kg)+PM(100mg/kg)	32.20±6.15[a,b]	1.02±0.28	0.004	S
4.HgCl(3.0mg/kg)+PM(200mg/kg)	37.96±7.78[a,b]	1.15±0.06[b]	0.001	S

UNDER PEER REVIEW

5.PM (100mg/kg)	45.92±9.39[a,b]	1.37±0.15	0.057	S
6.PM (200mg/kg)	102.69±19.81[a,b]	1.56±0.42	0.000	S

Each value represents the mean ± SD; [a]Significantly different from the control; [b]Significantly different from HgCl; ($p < 0.05$).
Key : CAT - Catalase, S - Significant. GPx - Glutathione peroxidase.

The results showed that there was elevation in liver MDA level in group 2 rats (Table 1), which reflects increased lipid peroxidation occurring in liver cells occasioned by mercury intoxication. One of the peroxidation products of lipid, MDA is an important indicator of radical oxidation of lipids have been reported to be highly increased in liver toxicity [13, 14]. This is in line with previous works submitted by Uzun and Kalender [15] and Kalender [16]. In PM seed treated groups 3 & 4 rats, there were significant reduction in liver MDA level (Table 1). This could be as a result of ameliorative influence of PM seed on liver cells, providing antioxidant capacity through scavenging and chelating processes [17]. The liver MDA levels in groups 5 & 6 rats respectively were greatly minimized, reflecting the ameliorative effects of PM seed extract. The activity of liver SOD in group 2 rats reduced significantly at the end of the experiment (Table 1). This could be due to mercury intoxication to liver cells inducing oxidative stress and generation of free radicals, causing cell injury and eventual death [18]. The elevation in liver SOD activities in PM seed treated groups 3 & 4 rats was remarkable, indicating ameliorative potentials of PM seed. This is also in line with the previous submission of some research teams on phytochemical, rutin. Rutin works as a scavenger of reactive oxygen species (ROS) and functions as a terminator and chelator of metal ions that are capable of oxidizing lipids [19, 20]. The significant decrease in liver GSH level in group 2 rats may be due to mercury intoxication to liver cells and tissues inducing oxidative stress and generation of free radicals (Table 1). Also the significant increase in liver GSH levels in PM seed treated groups 3 & 4 rats could be attributed to great ameliorative influence of PM seed extract. The liver GSH levels in groups 5 & 6 rats where only PM seed extract was administered at different concentrations were further enhanced by PM seed extract.

The reduction in liver CAT activity in group 2 rats reached significant level (Table 2), and could be as a result of increased oxidative stress – driven destruction of liver cells occasioned by mercury toxicity, resulting in the depletion of CAT activity and reducing its antioxidant capacity [18, 21]. The elevation in liver CAT activities in groups 3 & 4 rats upon treatment with PM seed at different concentrations reached significant levels. This could be as a result of great ameliorative potentials of PM seed extract on liver cells. This corresponds with the findings of some research scholars who reported on phytochemical, tannins. Tannins in the form of pro-anthocyanidins are useful as an anti – inflammatory agent and in the treatment of burns and wounds based on their anti – hemorrhagic, antioxidant and antiseptic. The liver CAT activities in groups 5 & 6 rats were further enhanced at the end of the experiment. There was significant decrease in liver GPx activity in group 2 rats where only mercury chloride was administered (Table 2). This could be as a result of mercury intoxication to liver cells provocating oxidative stress and lipid peroxidation. More so, the liver GPx activities in PM seed treated groups 3 & 4 rats recorded significant increase at the end of the experiment. The liver GPx activities in groups 5 & 6 rats were reasonably enhanced reflecting ameliorative capacity of PM seed extract which is greatly enriched with vital phyto – constituents such as, tannins, saponins, flavonoids, alkaloids, phenols, glycosides, polyphenolic compounds and rutins [22, 23].. These phytochemicals are known to have antioxidant potentials and are capable of mitigating the negative effects of free radicals and oxidative stress [24]. This agrees with previous submissions by some research teams who reported that most plants and natural products with antioxidant capacity have showed protection against mercury chloride – induced oxidative damage in experimental models [6, 25].

UNDER PEER REVIEW

The elevation in kidney MDA level in group 2 rats reached significant level (Table 3). This could be due to increased lipid peroxidation occurring in renal cells and tissues, precipitated by mercury intoxication and MDA an index of lipid peroxidation tends to rise during kidney toxicity. This corresponds with the findings of some research teams in their previous submissions on MDA as a specific biomarker for lipid peroxidation, who reported MDA rise during lipid peroxidation [13, 16]. The significant reduction in kidney MDA level in PM seed treated groups 3 & 4 rats could be as a result of ameliorative potentials exhibited by PM seed to renal cells and tissues. The kidney MDA levels in groups 5 & 6 rats were reasonably minimized by PM seed which is enriched with vital phytoconstituents [22, 23]. The decrease in renal GSH level in group 2 rats is significant (Table 3). This could be as a result of increased oxidative stress – driven destruction of renal cells and tissues, resulting to depletion of GSH concentration and reducing its antioxidant potentials. There were significant increase in kidney GSH levels in groups 3 & 4 rats upon treatment with PM seed at different concentrations, attributing it to ameliorative effect of PM seed extract. The kidney GSH levels in groups 5 & 6 rats where only PM seed extract was administered at different concentrations were further enhanced at the end of the experiment. The significant reduction in kidney SOD activity in group 2 animals may be due to mercury intoxication to kidney cells and tissues with increased oxidative stress and generation of free radicals (Table 3). This agrees with previous studies carried out by Ratston and his team in 2010 on mercury exposure to cellular selenium. High mercury exposure to kidney cells deplete the amount of cellular selenium available for the biosynthesis of thioredoxin reductase, GPx, SOD, and CAT, which, if the depletion is severe and long lasting, results in brain, liver, kidney and testicular cell dysfunctions that can ultimately cause death. The elevation in kidney SOD activity in PM seed treated groups 3 & 4 rats reached significant proportion and could be attributed to great ameliorative potentials of PM seed extract to renal cells and tissues. The kidney SOD activities in groups 5 & 6 rats were maintained at the end of the experiment.

The significant reduction in kidney CAT activity in group 2 rats could be as a result of mercury intoxication to kidney cells and tissues, inducing oxidative stress and lipid peroxidation (Table 4). This tallied with previous studies reported by some research teams who discovered that the kidneys are extremely susceptible to inorganic mercury deposition and toxicity [1, 5]. It was reported that mercury chloride induces biological toxicity in various tissues through multiple mechanisms including lipid peroxidation and oxidative stress which have been established to occur in the liver, kidney, testis and other tissues of experimental animals. There were significant elevation in kidney CAT activities in groups 3 & 4 rats on application of PM seed at different concentrations, attributing it to great ameliorative influence exhibited by PM seed extract on renal tissues and cells. The kidney CAT activities in groups 5 & 6 rats were strongly maintained by PM seed extract. There was significant decrease in kidney GPx activity in group 2 rats (Table 4). This could be due to increased oxidative stress – driven destruction of renal cells and tissues occasioned by mercury intoxication, resulting to depletion of GPx activity and decreasing its antioxidant potentials. GPx being a unique enzyme with peroxidase activity that protects cells from oxidative stress – driven destruction. Hydrogen peroxide and lipid peroxides are converted to water and alcohol respectively by [26]. There were significant increase in kidney GPx activities in groups 3 & 4 rats upon treatment with PM seed at different concentrations. This could be due to the ameliorative potentials of PM seed extract on kidney cells and tissues. The kidney GPx activities in groups 5 & 6 rats were maintained at the end of the experiment.

The elevation in testis MDA level in group 2 rats reached statistical significant level (Table 5). This could be attributed to increased lipid peroxidation occurring in testicular cells and tissues occasioned by mercury intoxication, generating free radicals and increasing production of MDA, an index of lipid peroxidation. This finding corresponds with previous submissions by some research teams who reported that increased MDA concentration is an

UNDER PEER REVIEW

important indicator of lipid peroxidation [15, 16]. MDA an index of lipid peroxidation is highly increased in liver, kidney and testicular toxicity [13, 14]. The significant reduction in testis MDA level in PM seed treated groups 3 & 4 rats could be due to great ameliorative influence exhibited by PM seed extract on testicular cells and tissues. MDA levels in groups 5& 6 rats were further reduced at the end of the experiment. The significant reduction in testis SOD activity in group 2 rats could be attributed to mercury intoxication, precipitating increased oxidative stress and generation of free radicals, causing death or injury to testicular cells and tissues, and finally depleting SOD activity (Table 5). This finding tallied with the report of Sarkar and his team [18] on ROS, which according to them, may induce cell injury or death and extreme production of ROS causes alterations of subcellular structures. Also, Stohs and Bagch [27], supported my findings in their report on effects of heavy metals on cells and tissues. Heavy metals such as, mercury, iron, cadmium, copper and arsenic can induce generation of reactive radicals and cause cell damage through depletion of enzyme activities, lipid peroxidation and reactions with nuclear proteins and DNA . More so, the significant increase in testis SOD activities in PM seed treated groups 3 & 4 rats could be due to remarkable ameliorative influence of PM seed extract on testicular cells and tissues (Table 5). Also, the testis SOD activities in groups 5 & 6 rats respectively were sustained and enhanced at the end of the experiment. The statistical significant decrease in testis GSH level in group 2 rats could be attributed to mercury intoxication resulting to oxidative stress and release of free radicals that caused reasonable damage to testicular cells and tissues. The significant increase in testis GSH level in groups 3 & 4 rats upon treatment with PM seed at different concentrations is attributed to great ameliorative impact of PM seed extract on testicular cells and tissues. Also, the testis GSH level in group 5 &6 rats were further improved.

The decrease in testis CAT activity in group 2 rats reached significant level, and could be as a result of mercury intoxication to testicular cells and tissues, resulting to increased generation of free radicals and ROS, leading to depletion of CAT activity and reducing its antioxidant potentials (Table 6). This corresponds to previous studies by some research teams who reported that heavy metals , such as, mercury, iron, copper, cadmium, lead and arsenic can induce generation of reactive radicals, and cause cellular damage through depletion of enzyme activities and lipid peroxidation [27]. Also, the statistical significant elevation in testis CAT activities in PM seed treated groups 3 & 4 rats could be due to ameliorative influence of PM seed extract on testicular cells and tissues. The testis CAT activities in groups 5 & 6 rats were enhanced and improved at the end of the experiment. There was significant decrease in testis GPx activity in group 2 rats (Table 6), and could be as a result of increased oxidative stress – driven destruction of testicular cells and tissues by mercury intoxication, resulting to depletion of GPx activity and reducing its antioxidant potentials. This is in agreement with previous submissions carried out by Bhabak and Mugesh [26] who reported GPx as a unique enzyme with peroxidase activity that protects cells from oxidative stress – driven destruction. Hydrogen peroxide and lipid peroxides are converted to water and alcohol by GPx. Also, there was significant elevation in testis GPx activities in PM seed treated groups 3 & 4 rats and in groups 5 & 6 rats both being attributed to great ameliorative influence of PM seed extract.

4. CONCLUSION

The results obtained from the study showed that mercury chloride has the potential to cause liver, kidney and testicular toxicity to adult male albino rats and this toxicity was exerted majorly through oxidative stress and lipid peroxidation. However, treatment with *Pentaclethra macrophylla* seed extract ameliorated the oxidative stress.

UNDER PEER REVIEW

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Zalups, R.K. Molecular interactions with mercury in the kidney. Pharmacological reviews, 2000; 52(1): 113-44.
2. Bernhoft, R.A. Mercury toxicity and treatment: a review of the literature. *Journal of environmental and public health*, 2012; 4:1-5.
3. Troen, P., Kaufman, S. A., & Katz, K. H. Mercuric bichloride poisoning. *New England Journal of Medicine*, 1951; 244(13): 459-63.
4. Aleo, MF., Morandini, F., & Bettoni, F. In vitro study of the nephrotoxic mechanism of mercury chloride. La medicina. Del Lavoro. 2002; 4(93): 267–78
5. Abarikwu, S. O., Njoku, R. C. C. & Onuah, C. L. Aged coconut oil with a high peroxide value induces oxidative stress and tissue damage in mercury-treated rats. *Journal of basic and clinical physiology and pharmacology,* 2018; 29(4): 365-76.
6. Abarikwu, S. O., Benjamin, S., Ebah, S. G., Obilor, G., & Agbam, G. Oral administration of Moringa oleifera oil but not coconut oil prevents mercury-induced testicular toxicity in rats. Andrologia, 2017; 49(1): *12597-9.*
7. Osabor, V. N., Okonkwo, P. C., & Ikeuba, A. I. Chemical profile of leaves and seeds of Pentaclethra macrophylla Benth. *Journal of Medicinal plant and Herbal Therapy Research,* 2017; 5: 11 – 17.
8. Handa, SS., Khanuja, SPS., Longo, G., & Rakesh, DD. Extraction Technologies for Medicinal and Aromatic plants, United Nations Industrial Development Organization and International Centre for science and High Technology, 2008 ; 66(8):1-6.
9. Ohkawa, H., Ohishi, N. & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 1979; *95*(2): 351-8
10. Moron, M. S., Depierre, J. W. & Mannervik, B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1979; *582*(1): 67-78.
11. Misra, H. P. & Fridovich, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 1972; *247*(10): 3170-5
12. Paglia, D. E., & Valentine, W. N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of laboratory and Clinical Medicine,* 1967; 70(1): 158–69.
13. Seppanen, K., Soininen, P., Salonen, JT., Lotjonen, S., & Laatikainen, R. Does Mercury promote lipid peroxidation? An in vitro study concerning mercury, copper,

UNDER PEER REVIEW

and Iron in peroxidation of low density lipo – protein. Biol Trace Elem Res, 2004; 01: 117–32.
14. Valko, M., Morris, H., & Cronin, MT. Metals, Toxicity and oxidative stress. Curr Med Chem, 2005; 12: 1161–208.
15. Uzun, F. G., & Kalender, Y. Chlorpyrifos – induced hepatotoxicity in rats and the protective role of quercetin and catechin. Food chem. Toxicol. 2013 55: 549–56.
16. Kalender, S., Apaydin, F. G., Uzunhiscarcikli, M., & Aslanturk, A. Protective role of sodium selenite and vitamin E against rat erythrocytes – induced by mercury chloride. *Gu. J. Sci.,* 2014; 27 (4), 1123–9.
17. Anpin Raja, R. D., Prakash, J. W., & Jeeva, S. Antibacterial activity of some medicinal plants used by Kani tribe, southern Western Ghats. India In: P. C. Trivedi, Editor. Ethnic Tribes and medicinal plants. Jaipur: Pointer publishers, 2010; 4: 28–45.
18. Sarkar, S., Mukherjee, S., Chattopadhyay, A., & Bhattacharya, S. Low dose of Arsenic trioxide triggers oxidative stress in zebrafish brain; Expression of antioxidant genes. Ecotoxicol. Environ, 2014; 107: 1–8.
19. Henry, F., Danoux, L., Pauly, G., & Charrouf, Z. A plant extract and its pharmaceutical and cosmetic use. Patent Applied WO. 2005.
20. Middleton, JE. Kandaswami, C., & Theoharides, TC. The effects of plant flavonoids on mammalian cells: implication for inflammation, heart disease, and cancer. Pharm Reviews, 2000; 52: 673– 51.
21. Clairborne A. (1995). Catalase activity. In: Greewald AR, editor. *Handbook* of methods for oxygen radical research (pp. 237–242) Boca Raton, FL: CRC Press;
22. Dongmo, F. G., Enyong, O. J., Noumessing, M. C., & Enyegue, M. D. Phytochemical constituents and Antioxidant potential of some Camroonian Medicinal Plants. Pharmacology on line, 2007; 2: 436–52.
23. Oyeleke, G. O., Odedeji, J. O., Ishola, A. D., & Afolabi, O. Phytochemical Screening and Nutritional Evaluation of African Oil Bean Seed (*Pentaclethra macrophylla*) IOSR. *Journal of Environmental Science, Toxicology and Food Technology,* 2014; 8: 14–17.
24. Okwu, D. E. Phytochemical, Vitamins and Mineral contents of Two Nigerian Medicinal plants. *International J. Molecular. Med. Adv. Sci.,* 2005; *1: 375–81.*
25. Unsal, V. Natural Phytotherapeutic Antioxidants in the Treatment of Mercury Intoxication-A Review. Advanced pharmaceutical bulletin, 2018; 8(3): 365-76.
26. Bhabak, K., P. & Mugesh, G. Functional mimics of glutathione peroxidase: bioinspired synthetic antioxidants. Accounts of Chemical Research, 2010; 43(11): 1408-19.
27. Stohs, SJ. & Bagch, D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med. 1995; 18: 321–36.

UNDER PEER REVIEW