Ethanol extract of M*yristica fragrans* (houtt) exhibits hepato-renal protective effects against lead-induced toxicity in Wistar rats

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ABSTRACT

Background: Lead is known to be a toxic substance which affects the liver, kidney and the brain by inducing oxidative stress owing to elevation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radicals and lipid peroxides. There is an increased interest among scientific investigators to use medicinal plants with antioxidant activity for protection against metal toxicity, especially lead toxicity. Therefore, the effect of ethanol extract of *myristica fragrans* (EEMF) on lead induced hepato-renal toxicity in rat was studied. *Methodology:* Thirty Wistar rats weighing 200-270g were distributed randomly into five groups. Group I(healthy control) was given normal saline (10ml/kg) for five weeks. Group II (Lead control) was given Lead acetate at a dose of 50mg/kg body weight for three weeks. Group III (Lead and *Myristica fragrans* co-treatment) received Lead acetate and *Myristica fragrans* extract at doses of 50mg/kg body weight and 150mg/kg body weight respectively. Group IV (*Myristica fragrans* pre-treatment) received *Myristica fragrans* extract at a dose of 150mg/kg body weight for two weeks followed by Lead acetate at a dose of 50mg/kg body weight for three weeks. Group V (*Myristica fragrans* post-treatment) was given Lead acetate (50mg/kg) for the first three weeks followed by *Myristica fragrans* (150mg/kg) for two weeks. Both Lead acetate and *Myristica frangrans* extract were administered orally. The levels of

the liver enzymes in the serum and markers to assay for kidney functions were measured. *Results:* The results showed that Lead significantly decreased body weight, liver antioxidant glutathione, total protein and increased alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, urea and creatinine when compared with the healthy control group $p \leq 0.05$. EEMF showed significant decrease ($p \leq 0.05$) in the levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, urea and creatinine while it significantly increased ($p \leq 0.05$) levels of glutathione and total protein when compared to the lead control group. There was a significant weight gain in the rats treated with *Myristica fragrans* (nutmeg). *Conclusion:* The data suggests that *Myristica fragrans* has protective effect against lead induced hepatorenal toxicity in Wistar rats.

Keywords: *Myristica fragrans*, Lead acetate, Hepatotoxicity, Nephrotoxicity, Liver enzymes, Kidney function markers, Wistar rat.

1. INTRODUCTION

Humans and animals directly or indirectly come in contact with toxic substances within their environment. Among these substances is Lead (Pb) which is a poisonous metal that occurs in the environment as either organic (Tetraethyl lead) or inorganic (lead chloride, lead acetate) (Shalan *et al*., 2005). Gidlow (2004) stated that inorganic lead is known to be one of the earliest occupational toxins discovered and that the facts about Lead poisoning was traceable to Roman times. Lead production started at least 5000 years ago and outbreaks of lead poisoning occurred from this time (Gidlow, 2004). Lead is a common cause of poisoning in domestic animals throughout the world (Khan *et al*., 2008). According to Graraza and co-workers lead has been implicated to be important in paintings, medicines, ammunition, pipes and recently in making alloys for welding utilities for preservation of chemical reagents (Garaza *et al*., 2006).

Lead (as an environmental pollutant present everywhere) is released into the atmosphere from production of iron, oil, coal, batteries, and steel. It also enters the atmosphere through smelting, smoking tobacco and solid wastes. Lead can cause biological disorders by changing the molecular interactions, cell signaling, and most importantly cellular function. Coutois et al, (2003) reported that Lead nitrate could produce a broad range of biochemical, physiological and behavioural dysfunctions in mammals due to its toxic effects. Lead exposure occurs mainly through the respiratory and gastrointestinal systems and the skin (Neathery, 2007). Martina and Krista wrote that, "the oral exposure to Lead is said to be more relevant to human environmental exposure" (Martina and Krista, 1987). Young children are prone to long time lead poisoning by sucking lead paint and playing with lead toys. According to Rossi (2008) exposure to Lead can take place by making contact with it in household dust, air, soil, water, and commercial products. Researchers have reported that Lead in air or soil could find its way into ground and surface water. In addition, Lead is found in drinking water obtained from plumbing pipes and fixtures manufactured from lead due to deposition of lead solder (Chisolm, 2004).

Moreover, Yücebilgic and co-woker said that "occupational and environmental exposures remain a serious problem in many developing and industrializing countries" (Yücebilgic *et al.,* 2003). Lead is known to be harmful even in minute amounts and the manifestations of its poisoning effects in humans are nonspecific. They may include weight loss, anemia (Khalil-Manesh *et al.,* 1994), infertility, testis and heart damages, nephropathy and hepatic destruction (Gurer-Orhan *et al,* 2004). Lead causes hepatic oxidative damage by favouring membrane lipids' peroxidation (Chaurasia and Kar, 1997), which is a harmful process produced only by free radicals (Halliwell and Gutteridge, 1990). Lead-induced oxidative stress in blood and other soft tissues has been postulated as one of the various possible ways by which lead could produce toxic effects (Pande *et al.,* 2001). To support this assertion, Sandhir and Gill, (1995) reported that lead could increase the level of lipid peroxides and also change antioxidant defense mechanism in the liver tissues. Reactive oxygen species (ROS) has been implicated in toxicity induced by lead (Gurer and Ercal, 2000). The toxic effect of lead on the liver tissues is connected with the disruption of the liver structure and function (Aziz *et al.,* 2012).

Interestingly today, people are shifting from the use of Orthodox medicines to that of traditional medicines derived from extracts of edible plants parts and its products owing to the fact that they are costly and produced undesirable harmful effects. Plants seem to be promising in providing an alternative means in the development of new drugs (Abalaka *et al.,* 2011). Herbal drugs analyze the part or parts of a plant used for the preparation of herbal and traditional medicine (for examples: leaves, seeds, flowers, roots, barks, stem etc) (Kayode and Kayode, 2011).

Antioxidants are reported to be actively involved to stop damage caused by free radical damage (Seifried *et al*., 2007). They are also believed to contribute to the beneficial effects through stimulating the antioxidative defense enzyme activities (Saha *et al*., 2004). There are many types of natural antioxidants. Polyphenols have been given much attention (Luo *et al*., 2002). Hence, the potential effects of flavonoids and phenolic acids as antioxidant agents in this category have been thoroughly studied (Rice-Evans *et al*., 1996; Shui and Lai, 2004).

Dietary consumption of fruits and vegetables contain abundant of natural ROS scavenging molecules including phenolic compounds (Shahidi and Naczk, 1995; Aruoma, 2003). A plant with the above properties and with the desired medicinal benefit is *Myristica fragrans* (Nutmeg).

Nutmeg is the dried seed kernel of *Myristica fragrans* plant widely used as a spice and also to flavour many kinds of baked foods and vegetables. Recent studies indicate that nutmeg is useful against damage caused by Cadmium sulphate (Bamidele et al, 2016), Gamma radiation and also in the improvement of mouse memory (Sharma and Kumar 2007; Parle *et al.,* 2004). Anti-inflammatory activity of nutmeg has also been reported in addition to its insulin-like biological activity (Olajide *et al.,* 1999; Broadhurst, 2000). There is paucity of information on the effects of *Myristica fragrans* seed on lead-induced hepatorenal toxicity in rats. In view of this, the present study was conducted to investigate the impact of ethanol extract of Myristica fragrans on lead induced hepatorenal toxicity in Wistar rats.

2. MATERIALS AND METHODS

Collection and extraction of plant materials

The nutmeg seeds (4kg) were purchased from Sango market, Ibadan, Nigeria. The Nutmeg seed was identified and authenticated by Dr Ayanbamiji of the Department of Biological Sciences, Bowen University, Iwo, Osun state (BUI/065). The nutmeg was shade dried after which were ground into powder by a manual hand grinder, yielding a weight of 2kg. Nutmeg powder was soaked in ethanol (70% v/v, BDH) for 72 hours and were stirred daily to ensure homogeneity. It was filtered at the 72nd hour using No 1 Whattman filter paper and the filtrate obtained was dried to a constant weight of 32g with a percentage yield of 1.6% using the rotary evaporator, and stored in refrigerator preserved with foil paper until use.

Experimental animals

Thirty (30) Wistar rats weighing 200-270g were obtained from Olu Research Farm, Sango, Oyo State. The rats were kept in cages in the Animal House of Department of Physiology, College of Health Sciences, Bowen University, Iwo, Nigeria under suitable conditions of temperature and humidity and provided with food and water ad-libitum. The rats were made to be accustomed to the Animal House for two weeks before beginning the experiment. The use and care of laboratory animals were carried out in accordance with the internationally accepted best practices as contained in the European Commission guidelines (EEC Directives of 1986; 86/609/EEC).

Experimental protocol

Thirty (30) Wistar rats were divided into five (5) groups, each group contains six rats. The experiment lasted for 5 weeks. The drugs route of administration was by oral gavage.

Group 1: the control group received normal saline from beginning of experiment until the 5th week.

Group 2: the lead control group received lead acetate daily for five weeks.

Group 3: the co- treatment group received lead acetate and *Myristica fragrans* simultaneously for five weeks.

Group 4: Pre-treatment group received *Myristica fragrans* for two weeks and lead acetate for three weeks. Group 5: Post treatment group received lead acetate for three weeks and *Myristica fragrans* for two weeks

Measurement of body weight of the rats

The body weights of the rats were measured before the start and at the end of the experiment. The body weight were measured using a sensitive weighing balance.

Blood collection

The rats were anaesthetized with chloroform, after sedation, the anterior wall of each of the rat was reflected to allow acess to the heart. Thereafter, blood was collected by cardiac puncture. The blood was left to clot in plain sample bottles for thirty (30) minutes which was later centrifuged at 5000rpm for ten (10) minutes and the serum was collected into plain sample bottles and the serum was used for the following analyses:

a) Assay for Liver functions: Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Glutathione (GSH)

b) Assay for Kidney functions: Total protein, Creatinine, Urea

Alkaline phosphatase (ALP)

Alkaline phosphatase was assayed for according to the Rec. GSCC method (1972). 0.02ml of serum was added to 1.0ml of the reagent and incubated for 40 minutes at 37°C. The reagent is made up of $MgCl_2$ buffer (0.5mmol/l diethanolamine) and 10mmol per litre of p-nitrophenylphosphate. It was read at the wavelength of 405nm initially then re-read after 1 and 2 minutes. The mean was taken and were expressed as U/L.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)

Activities of AST and ALT were determined by using Reitman and Frankel's method (1957). serum (0.1ml) was added to phosphate buffer(0.5ml) which contains L-alanine and α-oxaloglutarate (for AST) and mixed and incubated for 30 minutes at 37°C. To the result was added 0.5ml of 2,4-dinitrophenylhydrazine and allowed to stand for 20 minutes at 20-25°C. To the result, 5.0ml of 0.4mol/l sodium hydroxide was added and left for 5 minutes. The absorbance was read at 546nm.

Glutathione (GSH)

Glutathione was assayed for by the method used by Beutler and co-worker, (Beutler et al., 1963). The basic principle behind this method is the development of a relatively stable yellow colour when Ellman's reagent is mixed with sulphurhydryl agent. Few drops of the sample were added to equal volume of 4% sulphursalicyclic acid which was centrifuged at 4000rpm for 5 minutes. The supernatant (0.5ml) was added to Ellman's reagent (4.5mls). 40mg of Ellman reagent was dissolved in 0.1M phosphate buffer of pH 7.4 and made up to100mls. The phosphate buffer used was prepared by dissolving 0.496g of di-potassium hydrogen orthophosphate (K2HPO4) and 0.973g of potassium di-hydrogen orthophosphate (KH2PO4) in 9ml of distilled water. The pH was adjusted to 7.4 and then made up to 100ml with distilled water.

Creatinine, Urea and Total protein

Creatinine, urea, and total protein concentrations were measured using commercially available kits from Randox Laboratories Limited (United Kingdom) via spectrophotometry.

Statistical analysis

All the results from this present work were expressed as the mean ± SEM. The data were analyzed by analysis of variance (ANOVA) followed by Duncan test using the Graph pad prism. The level of significance was set at $p<0.05$ and $p<0.01$.

3. RESULTS

Body weight

The body weight of the rats in grammes was expressed in Mean ± Standard Error of Mean. The rats were weighed before the experiment (Week 0) and at the 5th week at the end the experiment. The result is shown in the table 1.

TABLE 1: Effect of Lead Acetate and *Myristica fragrans* on body weight female Wistar rats

GROUPS/ BODY WEIGHT (g)	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)
GROUP 1 Control	221.00±12.365	220.0±3.391
GROUP 2 Lead control	237.40±3.458	195.8±2.267[a]
GROUP 3 Co- Treatment	230.60±6.889	215.0±4.290[b]
GROUP 4 Pre- treatment	230.20±10.331	213.8±2.518[b]
GROUP 5 Post-treatment	239.00±8.444	243.4±2.581[ab]

a- Significant when compare with healthy control at $P<0.05$

b- Significant when compare with lead control at $P<0.05$

Effect of lead and *Myristica fragrans* on body weight

The body weight of the rats exposed to lead and *Myristica fragrans* is represented in table 1. When the body weight of Lead acetate control group (group 2) was compared with the healthy control group (group 1) there was a significant ($p<0.05$) decrease in body weight. When the *Myristica fragrans* post-treatment group (group 5) was compared to the healthy control group at $p<0.05$, there was a progressive increase in weight which is statistically significant. Comparison of *Myristica fragrans* co-treatment and pre-treatment groups with the healthy control group at $P<0.05$, showed a progressive decrease in body weight that was not statistically significant at $P<0.05$. There were significant increases in body weight in *Myristica fragrans* co-treatment, pre-treatment post-treatment groups when compared to the lead control group ($p<0.05$).

Effects of *Myristical fragrans* on lead induced changes in biochemical parameter

TABLE 2: Protective effect of *Myristica fragrans* against lead induced changes in some hepatic biochemical parameters in Wistar rats. (n=5)

GROUP	AST (U/L)	ALT (U/L)	ALP(U/L)	GSH(U/L)
GROUP 1 Healthy control	31.440± 10.490	20.800±2.568	115.4±5.119	21.76±6.006
GROUP 2 Lead control	78.20±13.16^{ap}	56.780±1.320^{ap}	183.3±15.43^{ap}	12.450±2.504^{ap}
GROUP 3 Co-treatment	51.980±11.62	38.720±9.306	106.0±19.89^{bq}	29.81±8.263
GROUP 4 Pre treatment	23.41±6.989^{bq}	53.760±4.109^{ap}	154±13.05	67.22±9.159^{abpq}
GROUP 5 Post treatment	21.26±6.583^{bq}	35.59±9.306	129.7±19.08	71.34±10.77^{abpq}

a= p value at 0.05 when compared to the healthy control group (group 1)
b= p value at 0.05 when compared to the lead exposed rats (group 2)
p= p value at 0.01 when compared to the healthy control group (group 1)
q= p value at 0.01 when compared to the lead exposed rats (group 2)

Effects of lead and Myristica fragrans on some hepatic and renal biochemical parameters

Some hepatic biochemical parameters in Wistar rats treated with lead acetate and *Myristica fragrans* is represented in table 2 and figures 1, 2, 3. The biochemical parameters measured include alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Glutathione (GSH), Total Protein (TP), Creatinine Kinase (CK) and Urea (U).

Alkaline Phosphatase (ALP)

The comparison of lead group (Group 2) in relation to healthy control group (Group 1) showed a significant increase in ALP ($p<0.05$ and $p<0.01$ respectively). When co-treatment group (group 3), pre treatment group (group 4) and post treatment group (group 5) were compared to the healthy control group (group 1), there was no significant difference. When co-treatment group (group 3), pre treatment group (group 4) and post treatment group (group 5) were compared to the lead control group (group 2), only co-treatment group statistically decreased ALP level ($p<0.05$ and $P<0.01$ respectively).

Aspartate Aminotransferase (AST)

When lead control group (group 2) was compared to the healthy control group (group 1), there was an increase in AST level that is statistically significant ($p<0.05$ and $p<0.01$ respectively). Comparison of *Myristica fragrans* co-treatment, pre treatment and post treatment groups to the healthy control group showed no statistical difference. There were significant decrease in AST level in pre-treatment (Group 4) and post treatment (Group 5) groups only when compared to the lead control group (Group 2) as shown in Table 2.

Alanine Aminotransferase (ALT)

When lead control group (group 2), co treatment group (group 3), pre treatment group (group 4) and post treatment group (group 5) were compared to the healthy control group (group 1) at $p<0.05$ and 0.01, there were significant increases in ALT level in the lead

control and the *Myristica fragrans* pre treatment groups ($p<0.05$ and $p<0.01$). There was reduction in AST level in the *Myristica fragrans* co-treatment, pre treatment and post treatment groups but statistically significant.

Glutathione (GSH)

There was a significant reduction in GSH in the lead treated group (group 2) when compared to the healthy control group (group 1) while *Myristica fragrans* pre treatment and post treatment groups showed statistical increase at $p< 0.05$ and 0.01. The *Myristica fragrans* pre treatment and post treatment groups (groups 4 and 5) increased GSH significantly when compared to the lead control group (group 2) at $p<0.05$ and 0.01 (shown in table 2).

Urea

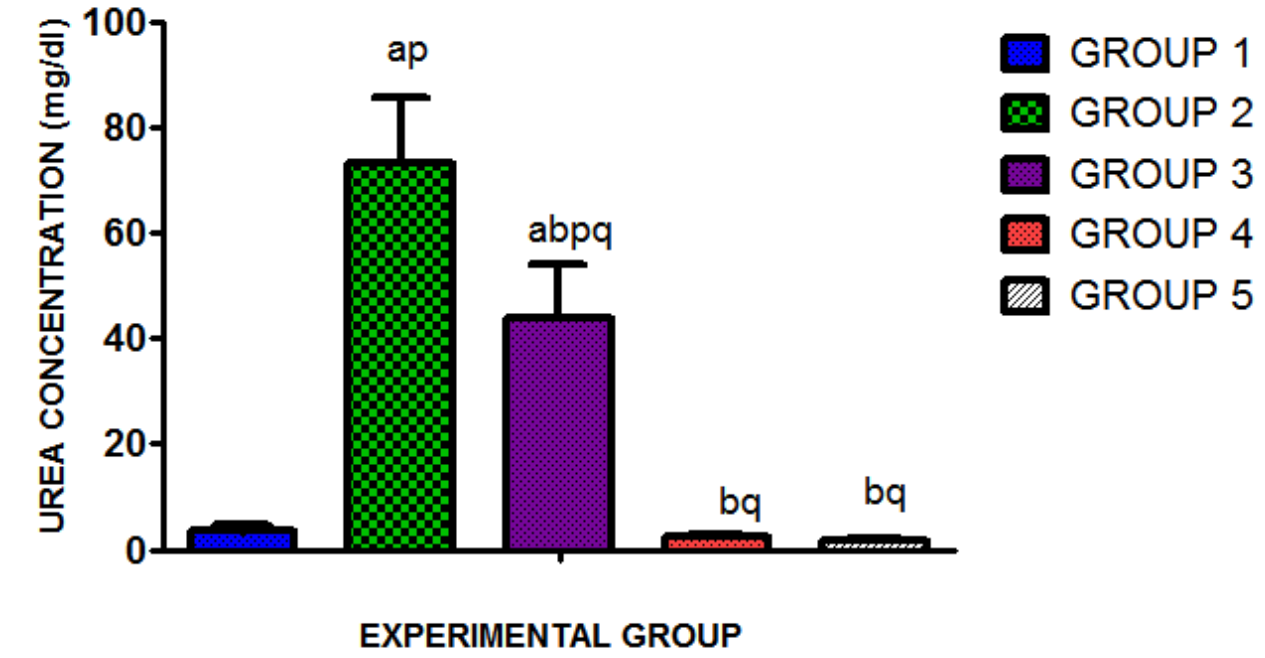

FIGURE 1: CHART SHOWING UREA LEVEL OF HEALTHY CONTROL AND LEAD TREATED GROUPS WITH OR WITHOUT *MYRISTICA FRAGRANS* IN THE EXPERIMENTAL RATS.

a- Significantly different from the healthy control group (group 1) at $p<0.05$
b- Significantly different from lead control group (group 2) at $p<0.05$
p- Significantly different from the healthy control group (group 1) at $p<0.01$
q- Significantly different from lead control group (group 2) at $p<0.01$

The effects of lead and *Myristica fragrans* on urea concentration in rats is shown in figure 1. Lead control group (group 2) significantly increased urea concentration in the experimental animal when compared to healthy control group (group 1) at $p<0.05$ and 0.01. Also, a significant increase was observed in co treatment group (group 3) when compared to healthy control group (group 1) at $p<0.05$ and 0.01. Statistical decreases were observed in co-treatment (group 3), pre treatment (group 4) and post treatment groups (group 5) compared to the lead control group ($p<0.05$ and $p<0.01$ respectively).

Creatinine Kinase

In figure 2, the lead control group (group 2) and co treatment group (group 3) showed no significant difference in creatinine level from healthy control group while pre treatment (group 4) and post treatment (group 5) groups statistically decreased creatinine level when compared to the healthy control group (group 1) at $p< 0.05$ and 0.01. When the *Myristica fragrans* pre treatment and post treatment

groups (groups 4 and 5) were compared to the lead control group (group 2), there were decreases in creatinine levels that are statistically significant (p<0.05 and P<0.01 respectively).

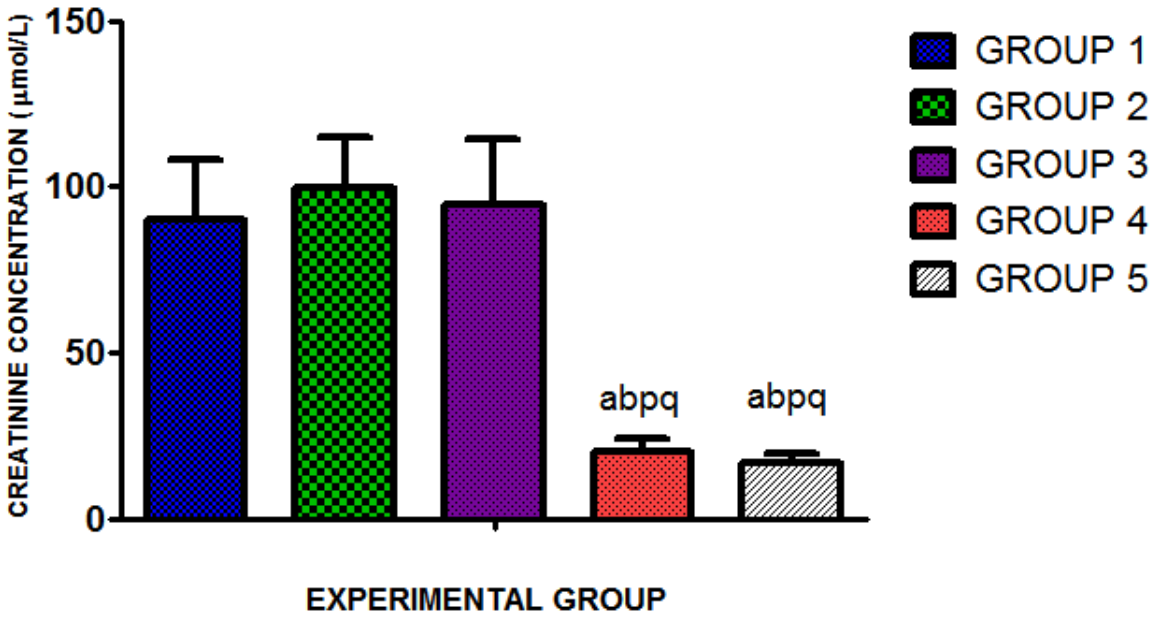

FIGURE 2: CHART SHOWING CREATININE KINASE LEVEL OF HEALTHY CONTROL AND LEAD TREATED GROUPS WITH OR WITHOUT *MYRISTICA FRAGRANS* IN THE EXPERIMENTAL RATS.

a- Significantly different from the healthy control group (group 1) at p<0.05

b- Significantly different from lead control group (group 2) at p<0.05

p- Significantly different from the healthy control group (group 1) at p<0.01

q- Significantly different from lead control group (group 2) at p<0.01

Total protein

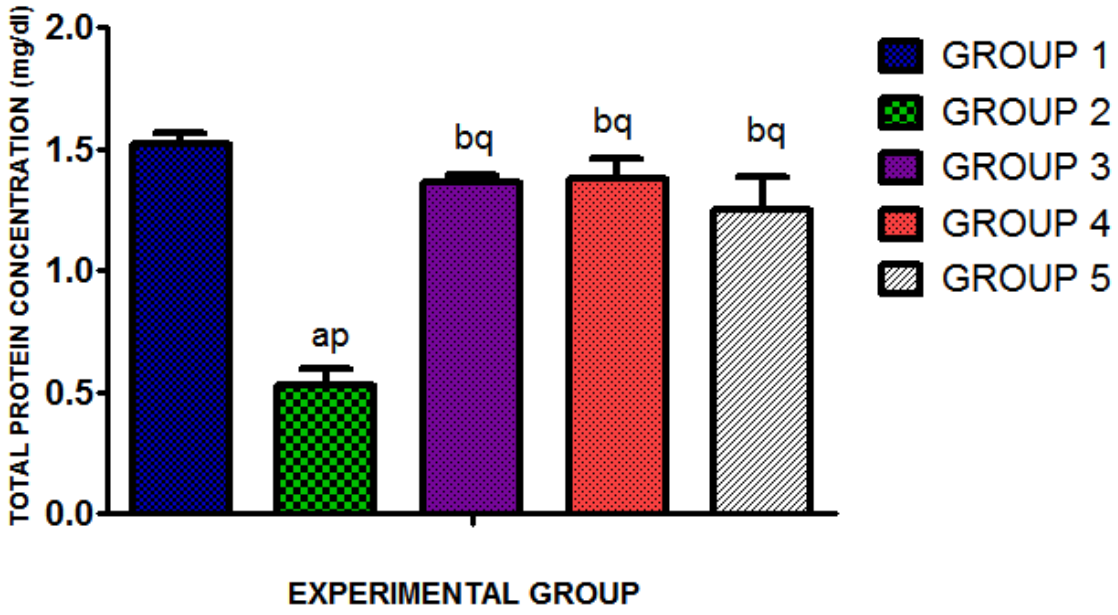

FIGURE 3: CHART SHOWING TOTAL PROTEIN LEVEL OF HEALTHY CONTROL AND LEAD TREATED GROUPS WITH OR WITHOUT *MYRISTICA FRAGRANS* IN THE EXPERIMENTAL RATS.

a- Significantly different from the healthy control group (group 1) at p<0.05

b- Significantly different from lead control group (group 2) at $p<0.05$

p- Significantly different from the healthy control group (group 1) at $p<0.01$

q- Significantly different from lead control group (group 2) at $p<0.01$

In figure 3, when the lead control group (group 2) was compared to the healthy control group at $p<0.05$ and 0.01, there was a decrease in total protein level that is statistically significant. Group 3, 4 and 5 showed no significant difference in total protein level from the healthy control. When co treatment group (group 3), pre treatment group (group 4) and post treatment group (group 5) were compared to the lead control group (group 2), there were increases in total protein levels that are statistically significant ($p<0.05$ and $P<0.01$ respectively).

4. DISCUSSION

Lead is a toxic heavy metal known to affect many organs in the body. Liver and kidney are frequent target for many toxicants (Meyer and Kukerni, 2001). Toxicological studies showed that the liver is the largest location for storage of Lead in humans among soft tissues. This followed by kidney. This has also been shown by various works done in mammals. Due to this, the present study was investigated to evaluate the effects of EEMF on lead-induced hepatorenal toxicity in Wistar rats.

The animals in the lead control group experienced weight loss which was statistically significant at the end of experimental week when compared to the healthy control group. The weight loss might be due to the decreased water and food intake and exposure of the animal to Lead (Nivetha and Prasanna, 2014; Bamidele et al, 2015; Nwokocha et al, 2012). Lead is implicated to reduce protein synthesis possibly by damaging DNA and RNA (Shalan et al, 2005) and this may be connected with base pair mutation, deletion and free radical attack. The animals treated with EEMF showed a significant weight gain when compared to the lead control group. This is in consonance with the study carried out by Nivetha and Prasanna where Myristica fragrans reversed weight loss caused by Gentamicin-induced toxicity (Nivetha and Prasanna, 2014).The weight gain was due to the changes in nutrient absorption and metabolic utilization.

Determination of biochemical parameters has been generally accepted as a biological indicator of liver impairment. In the rats treated with lead acetate alone there was a significant increase in the AST, ALT and ALP when compared to the rats in the healthy control group. Usually when there is hepatotoxicity, enzymes like alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase show a significant increase in their levels (Cornelius, 1979). There were reductions in the levels of these enzymes in the rats treated EEMF when compared to the lead control group. The reductions in the levels of these enzymes is in agreement with the work of Kareem *et al.,* 2013 who worked on the hepatoprotective effect of nutmeg on Isoprenoid induced toxicity and concluded that nutmeg ameliorate hepatotoxicity in rats.

Glutathione is usually regarded as the body's master antioxidant. It is found in every cell of the body with the highest concentration in the liver. Their level is usually decreased in liver toxicity in the liver and serum (Bechara, 2004). There was a significant decrease in glutathione in the lead control group, this occurred due to the inhibition of the antioxidant activity of glutathione (Dickinson *et al*., 2003). The rats treated with EEMF both pre treatment and post treatment groups showed significant increase in serum glutathione level. This increase occurs due to the antioxidant property of nutmeg. This result is in agreement with the research work carried out by Sharma *et al.,* 2010 with the use of garlic as the antioxidant treatment.

Total protein assay estimates the level of proteins in the body which include: albumin and globulin. There is usually decreased serum total protein when there is a compromise to the glomerular filtration of the kidney. When the lead control group was compared to the healthy control group, there was a decrease in total protein level that was significant. Treatment with EEMF (post treatment and pre treatment) in group 4 and 5 significantly increased total protein level when compared to the lead control group. This result corresponds to that which was reported by Nivethar and Prasanna (2014) on the work carried out to evaluate the effect of *Myristica fragrans* against gentamicin induced nephrotoxicity in albino rats.

Urea and creatinine are kidney function tests carried out to determine the functioning state of the kidney. Hepatotoxicity causes the significant increase in the levels of these enzymes. Rats treated with lead alone showed a significant increase in the level of these enzymes which may result from protein breakdown. Rats post treated and pre treated with EEMF showed significant decrease in the level of these enzymes. These results are in accordance with the result of the work done by Nivethar and Prasanna, 2014. These results showed that EEMF has ability to protect the kidney from chemical toxins such as lead.

5. CONCLUSION

The results of this study have shown that extract of nutmeg's seed is effective in protecting and ameliorating the damage done by lead in liver and kidney tissues. Therefore, addition of nutmeg in the human daily diet should be encouraged, most especially in the populations at risk of lead poisoning due to prolonged environmental exposure.

Authors' Contributions

This work was carried out in collaboration by all authors. Author OB designed the study, wrote the manuscript and interpreted the data. Authors, DSA participated in experimental design, bench work and data interpretation. Authors LDA, OOO and AOO participated in the bench work managed the literature searches and reviewed the manuscripts. Author OA carried out lead induced hepatorenal toxicity procedures and managed the literature searches and the analysis of data. All authors read and approved the final manuscript.

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

Peer-review:

External peer-review was done through double-blind method.

Data and materials availability:

All data associated with this study are present in the paper.

REFERENCE

1. Abalaka M, Oyewole OA, Kolawole AR. Antibacterial Activities of Azadirachta Indica against Some Bacterial Pathogens. *Journ. Mic. Res.* 2011: 1(2): 6-8.
2. Arouma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in food plants. *Mut. Res.* 2003:(523-524):9-20.
3. Aziz FM, Maulood IM, Chawsheen MAH. Effects of melatonin, vitamin C and E alone or in combination on lead-induced injury in liver and kidney organs of rats. IOSR J. Pharm. 2012: 2(5): 13-18.
4. Bamidele O, Adebiyi IT**,** Babatunde LD and Arokoyo DS. The Effect of Ethanolic Extract of *Basella alba L*. Leaves on Lead Induced Hepatotoxicity in Male Albino Wistar Rats (*Rattus norvegicus*), Interrnational Journal of Plant Science and Ecology. 2015: 1(5): 218-224.
5. Bamidele O, Orti D, Babatunde LD, Adedeji GT, Akinnuga AM, Arokoyo DS. Ethanolic extract of *Myristica fragrans (houtt*) seeds ameliorates cadmium-induced hepatotoxicity and nephrotoxicity in female wistar rats. Der Pharmacia Lettre. 2016: 8 (6):77-86.
6. Bechara EJH.Lead poisoning and oxidative stress. Free Radic. Biol. Med. 2004: 36:S22.
7. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 1963: 61:882-888.
8. Broadhurst CL, Polansky MM, Anderson RA. Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vivo. J. Agric. Food Chem. 2000: 48(3):849-52.
9. Chaurasia SS, Kar A. Protective effects of vitamin E against lead induced deterioration of membrane associated type-1 iodothyronine 5-monodeiodinase (5D-I) activity in male mice. Toxicol. 1997:124:203-209.
10. Chisolm JJ. 'Lead Poisoning'. In Crocetti M, Barone M.A, Oski F.A. Oski's Essential Pediatrics, 2nd Edition. Lippincott Williams and Wikins. 2004. ISBN 0-7817-3770-2.
11. Cornelius CE. Biochemical evaluation of hepatic function in dogs. Journal of American Animal Hospital Association. 1979: 15: 25-29.
12. Courtois E, Marques M, Barrientos A. Lead-induced downregulation of soluble guanylate cyclase in isolated rat aortic segments mediated by reactive oxygen species and cyclooxygenase- 2. J. Am. Soc. Nephrol. 2003:14: 1464-70.
13. Dhingra D, Sharma A. Antidepressant-like activity ofn-hexane extract of nutmeg (*Myristica fragrans*) seeds in mic. J. Med. Food. 2006: 9(1): 84-89.
14. Dickinson DA, Moellering DR, Iles E, Patel RP, Levonen AL and Wigley A. Cytoprotection against oxidative stress and the regulation of gluthione synthesis. *Biol. Chem.* 2003:384:527-537.
15. Ellman GL. Tissue sulphydryl groups. Arch. Biochem. Biophys. 1959: 82:70-7.
16. Garaza AR, Vega Soto E. Cellular mechanisms of lead neuro toxicity. Med. Sci. Monitor. 2006:12(3): 57-65.
17. Gidlow DA. Lead toxicity. Occup. Med. 2004: 54: 76-81.
18. Gurer-Orhan H, Sabir HU, Ozgüne H.Correlation between clinical indicators of lead poisoning and oxidative stress

parameters in controls and lead-exposed workers. Toxicol. 2004: 195:147-154.
19. Gurer H, Ercal N. Can antioxidant be beneficial in the treatment of lead poisoning? Free Radic. Biol. Med. 2000: 29: 927-945.
20. Halliwell B, Gutteridge JMC. Free radicals and catalytic metal ions in human disease: an overview. Meth. Enzymol. 1990: 186:1-85.
21. Kayode AA, Kayode OT. Some Medicinal Values of Telfairia occidentalis: A Review. *American Journal of Biochem. and Mol. Bio.* 2011:1:30-38.
22. Kareem MA, Gadhamsetty SK, Shaik AH, Prasad EM, Kodidhela LD. Protective effect of nutmeg aqueous extract against experimentally-induced hepatotoxicity and oxidative stress in rats. J. Ayurveda Integr. Med. 2013: 4:216-23.
23. Khalil-Manesh F, Tartaglia-Erler J, Gonick HC. Experimental model of lead nephropathy. Correlation between renal functional changes and hematological indices of lead toxicity. J. Trace Elem. Electrolytes Health Dis. 1994: 8:13-19.
24. Khan MS, Mostafa MS, Hossain MA, Sayed MA, Bang J. Effect of garlic and vita-min B-complex in lead acetate induced toxicities in mice. Vet. Med. 2008: 6(2): 203-210.
25. Kim R, Rotnitsky A, Sparrow D, Weiss S, Wager C, Hu H. A longitudinal study of low-level lead exposure and impairment of renal function. The Normative Aging Study. *JAMA*. 1996: 275: 1177-81.
26. Luo XD, Basile MJ, Kennelly EJ. Polyphenolic antioxidants from the fruit of *Chrysophyllum cainito* L. (Star Apple). Journ of Agricultural and Food Chem. 2002: 50: 1379-1382.
27. Martina P, Krista K. Bulleting of Environmental Contamination and Toxicol. 1987:39(3): 448-452.
28. Meyer SA, Kulkarni AP. Hepatotoxicity. In: Hodgson E, Smart RC editors. *Intr. Biochem. toxicol.* New York: John Wiley. 2001: 3: 487-490.
29. Neathery MW, Miller WJ. Metabolism and Toxicity of Cadmium, Mercury, and Lead in Animals: A Review. J. Dairy Sci. 2007):58(12).
30. Nivetha J, Prasanna G. Nephroprotective Effect of Myristica fragrans Against Gentamicin-induced Toxicity in Albino rats. Int. J. Adv. Pharm. Sci. 2014:5(3):2039-2045.
31. Nwokocha CR., Nwokocha MI, Owu DU, Obi J, Olatunde B, Ebe C, Nwangwu O, Iwuala MO. Comparative analysis on the effect of palm oil (Elaeis guineensis) in reducing cadmium and lead accumulation in liver of Wistar rats. Pharmacognosy Research. 2012: 4(4): 214-218.
32. Olajide OA, Ajayi FF, Ekhelar AI, Awe SO, Makinde JM, Alada AR. Biological effects of Myristica fragrans (Nutmeg) extract. Phytother Res. 1999:13:344-345.
33. Pande M, Mehta A, Pant B, Flora SJ. Combined administration of a chelating agent and an antioxidant in the prevention and treatment of acute lead intoxication in rats. Environ. Toxicol. Pharmacol. 2001: 9:173-184.
34. Parle M, Dhingra D, Kulkarni SK. Memory strengthening activity of Glycyrrhiza glabra in exteroceptive and interoceptive behavioural models. J med food. 2004): 7(4):462-6.
35. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol. 1957: 28:56-63.
36. Rec Gscc (DGKC). Optimised standard colorimetric methods. J.Clin. Chem. Clin. Biochem. 1972: 10: p. 182.
37. Rice-Evans CA, Miller NJ, Paganga G. Structure-Antioxidant Activity Relationships of Flavonoids and Phenolic acids. Free Radic. Biol. Med. 1996: 20:933-956.
38. Rossi E. "Low level environmental lead exposure - a continuing challenge". The Clinical biochemist. Reviews / Australian Association of Clin. Biochem. 2008: 29(2):63-70.
39. Saha K, Lajis NH, Israf DA, Hamzah AS, Khozirah S, Khamis S and Syahida A. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. J. Ethnopharmacol. 2004: 92:263-267.
40. Sandhir R, Gill K. Effect of lead on lipid peroxidation in liver of rats. Biol. Trace Elem. Res. 1995:8:91-97.
41. Seifried HE, Anderson DE, Fisher EI, Milner JA. A review of the interaction among dietary antioxidants and reactive oxygen species. J. Nutr. Biochem. 2007:18(3):168-71.
42. Shahidi and Naczk. Food phenolics: Sources, Chemistry, Effects, Applications. 1995: p 231-245.
43. Sharma M, Kumar M. Radioprotection of swiss albino mice by *myristica fragrans* houtt. J. Radiat. Res. 2007: 48:135-41.
44. Sharma A, Sharma V, and Kansal L. Amelioration of lead-induced hepatotoxicity by Allium sativum extracts in Swiss albino mice. Libyan journal of Medicine: 2010: 5: 4621.
45. Shalan MG, Mostafa MS, Hassouna MM, El-Nabi SE and El-Refaie A. Amelioration of lead toxicity on rat liver with vitamin C and silymanrin supplements. *Toxicol.* 2005: 206: 1-15.
46. Shui G, Lai P. An improved method for the analysis of major antioxidants of *Hibiscus esculentus* Linn. Journal of Chromatography. 2004:1048: 17-24.
47. Yücebilgic G, Bilgin R, Tamer L, Tükel S. Effects of lead on Na^+-K^+ ATPase and Ca^{2+} ATPase activities and lipid peroxidation in blood of workers. Int. J. Toxicol. 2003: 22:95-97.