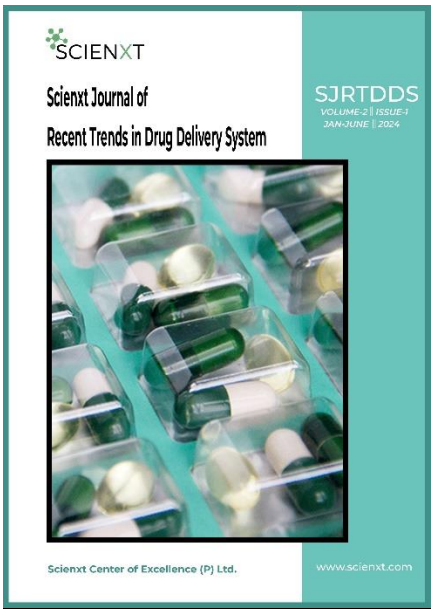

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Preventive effect of the whole plant aqueous extract of eleusine indica(Linn)gaertn. extract (Poaceae) against mercuric chloride-induced hepato- nephrotoxicity in rat

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Abstract:

The entire plant of *Eleusine indica* is used in Cameroonian folk medicine to treat several diseases including renal and hepatic disorders. The aim of this study was to evaluate the preventive effects of *Eleusine indica* aqueous extract against mercuric chloride induced- hepatic and renal damages in rats. Animals were divided into a normal control group, receiving 0.9 % NaCl subcutaneously (s.c) at the dose of 10 mL/kg, a negative control group receiving $HgCl_2$ (0.02 mg/kg, s.c) and three others groups receiving per os the verapamil (0.5 mg/kg) or the plant extract (100 or 200 mg/kg) simultaneously with $HgCl_2$. After 30 days of treatment, animals were sacrificed. The blood was collected for the assessment of the serum activities of ALT, AST and ALP, serum levels of total bilirubin, total proteins, albumin, lipid profile parameters, creatinine, urea, uric acid, Na^+ and K^+. MDA, SOD, catalase and GSH levels were measured in liver and kidney. $HgCl_2$ induced marked hepatotoxicity as evidenced by significant elevation in serum levels of ALT, AST, ALP, total bilirubin, total cholesterol, triglycerides and LDL, with significant reduction of HDL, total proteins and albumin as compared to controls, while nephrotoxicity was evidenced by significant elevation in serum levels of creatinine, urea, uric acid and K^+, with significant reduction of Na^+ as compared to controls. MDA was significantly increased, when SOD, catalase and GSH were significantly decreased in $HgCl_2$ injected groupsas compared to controls. *Eleusine indica* aqueous extract prevented various modifications of biochemical and oxidative markers. This study shows that *Eleusine indica* aqueous extractprevents $HgCl_2$ induced-hepato-nephrotoxicity, probably due to its antioxidant activities. These results justify the traditional use of this plant in the management of kidneys and liver problems.

Keywords:

Mercury, *Eleusine indica*, hepatotoxicity, nephrotoxicity, rat

1. Introduction:

Mercuric chloride ($HgCl_2$) is a hazardousenvironmental and industrial mercury toxic, which induces severe alterations in the body tissues of both humans and animals. Thesealterations result in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive anddevelopmental disorders. Nowadays, large populations worldwide are exposed to relatively low levels of mercury (Hg), especially via the use of pesticides in agriculture and as well as fluorescent lightbulbs[3]. The toxicity of mercury depends on the forms of the mercury compounds (elemental, inorganic and organic). Exposure to Hg vapor as well as to organic Hgcompounds specifically affects the central nervous system, while kidneys, liver and gastrointestinal tract are mainly targeted by inorganic Hg compounds, such as mercuricchloride.

Mercuric chloride administration is a classic model for the study of the pathogenesis of inorganic mercury toxicity in both *in vitro* and*in vivo* systems $HgCl_2$ affects the oxidative function due to its high affinity for cellular cysteine thiols. Mercury nephrotoxicity is characterized by altered antioxidant enzymes, lipid peroxidation, reduced ATP content which leads to tubular epithelium necrosis even after a single exposure. $HgCl_2$-induced damage is strictly dependent on the route of administration, time and dose. High Hg exposure causes an increase in free radicals production, thus induces oxidative stress which is involved in the pathogenesis of acutehepato-renal disorders. Inorganic Hg toxicity of the liver and kidney has been related to its binding to endogenous thiol-containing molecules. Considering that oxidative stress and endogenous thiol depletion are involvedin mercury chloride toxicity, it has been suggested that antioxidants could contribute to the treatment of Hg poisoning. In thisway, *Eleusine indica* has been found to possess antioxidant activity in CCl4-mediatedoxidative hepatic damage in rats.

Eleusine indica or Wiregrass (grass Poaceae family) is a native plant of the tropics and subtropical regions. The whole plant, especially the roots, is used in traditional medicine as a diuretic, anti-helminthic, diaphoretic, febrifuge and for treating cough.The decoction is consumed as anti-helminthic and febrifuge treatments. The seedis sometimes used in the treatment of liver disorders. Studies have shown that C- glycosylflavones from *Eleusine indica* haveanti-inflammatory effects onlipopolysaccharide-induced lung airwayinflammation in mice. The infusion of aerialparts of *Eleusine indica* is used in Brazil against airway inflammatory processes, such as pneumoniaInformation provided by traditional healers in Center Region of Cameroon indicates that the whole plant of *Eleusine indica* is used in the management of renal problems. Thus, the

present study was designed to evaluate the hepato- nephroprotective effects of the aqueous extract of *Eleusine indica* in mercuric chloride induced hepato-nephrotoxicity in rats.

2. Material and methods preparation of plant extract:

The whole plant of *Eleusine indica* was collected from Ngoa-Ekelle in the center region of Cameroon in January 2015. The plant material was authenticated at the National Herbarium, Yaounde by Mister NGANSOP TCHATCHOUANG Eric, in comparison to a sample N° 8356 SRF/CAM (YA). The whole fresh plant was washed thoroughly tap water, air dried at room temperature and reduced in powder. The powder (300 g) was boiled in 5L of tap water during 20 minutes according to the traditional healer's instructions. The mixture was filteredwith Whatman N◦ 3 filter paper. The solution obtained was evaporated at 45°C in dryingcupboard and gave 15.8 g of the aqueous extract (yield 5.27 %).

2.1. Phytochemical profile:

Phytochemical analyses of the aqueous extract were done following the procedure described by Sofowora and Ayoola *et al*. The chemical groups tested were alkaloids,saponins, flavonoids, cardiacglycosides,phenols, lipids, sugars and tannins.

2.2. Animal:

Twenty five male Wistar rats of 12 weeks old,weighting 140-190 g were obtained from the animal house of the Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1, Cameroon. Animalswere maintained under standard laboratory conditions with natural luminosity cycle, withfree access to normal laboratory rat food and tap water. All procedures in this study followed the principles of laboratory animal use and care of the "European community" guidelines (EEC Directive 2010/63/EEC) and were approved by the "Animal Ethical Committee" of the Faculty of Science, University of Yaounde I.

2.3. Animals grouping and treatments:

After 2 weeks of acclimatization, all animals were randomly divided into five groups offive rats each: group I served as control and was treated subcutaneously (s/c) with 0.9 % NaCl simultaneously with distilled water(orally at the dose of 10 mL/kg) ; Group II (Hg-treated) was given a single injection (s/c)of mercuric chloride ($HgCl_2$) at the dose of 0.02 mg/kg simultaneously

with distilled water (orally at the dose of 10 mL/kg) ;Group III (Vera + Hg-treated group) was given asingle injection (s/c)of$HgCl_2$at the dose of 0.02 mg/kg simultaneously with verapamil solution (orally at the dose of 0.5 mg/kg) ; Group IV and V (Ext. + Hg-treated group) were given a single injection (s/c)of $HgCl_2$(0.02 mg/kg) simultaneously with*Eleusine indica* aqueous extract orally at respective doses of 100 and 200 mg/kg.

All groups received the treatment once a day for 30 days. Body weight was recorded at the beginning and the end of the experimentalperiod.

2.4. Assessment of liver and kidney functions:

Twenty-four hours after mercury last injection, rats were anesthetized with urethaneand sacrificed. The carotid arteries blood was collected from carotid arteries into clean dry test tubes. Serum was separated by centrifugation (3000 rpm at 4° C for 15 min) and collected for the determination of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total cholesterol and HDL-cholesterol (HDL-c) using Inmesco kit, triglycerides and uric acid using SGMitalia kit, alkaline phosphatase (ALP),total bilirubin, creatinine and ureausing Fortress kit, sodium (Na^+) and potassium (K^+) using SEPTRUM kit according to the instructions provided by the manufacturer. Total proteins were alsoevaluated using the method of Gornall *et al.*The level of LDL-cholesterol (LDL-c) was determined using the formula:

LDL-cholesterol (mg/dL) = total cholesterol - (triglycerides/5) – HDL-c according to the commercial diagnostic kit Inmescoindications.

2.5. Assessment of hepato-renal oxidative stress:

Homogenate (20%) of liver and kidney were prepared in Tris-HCl buffer solution (pH 7.4).Organs were crushed and then the mixturewas centrifuged at 3000 rpm at 4° C for 25 min. The supernatant was collected and storedat -20° C until tissue analyses.Malondylaldehyde (MDA) was determined using the procedure of Wilbur *et al.* Superoxide dismutase (SOD) was determined using the method described by Misra and Fridovich. Catalase was determined according to Sinha whereas reduced glutathione (GSH) was determined using the method described by Ellman.

2.6. Statistical analysis:

Data were expressed as mean ± standard errorof mean. Statistical analysis was performed using one-way analysis of variance (ANOVA)followed by the Tukey post hoc test. $p<0.05$ was

considered statistically significant. All analyses were performed using Graphpadprism software 5.03 version.

3. Results Phytochemistry profile:

The aqueous extract of *Eleusine indica*contained primary as well as secondary metabolites. Alkaloïds, saponins, flavonoïds, cardiac glycosides, phenols and tanninswere present, whereaslipidsand sugars were absent.

3.1. Effects of *Eleusine indica* aqueous extract on body weight:

The variations of body weight of rats during the period of treatment are shown in Table. 1. The administration of $HgCl_2$during thirty daysis characterized by a significant weight loss ($p<0.05$) throughout the experimental period as compared to controls. This weight loss was by 87.35 %.However, the administration of the plant extract at both doses (100 and 200 mg/kg) with $HgCl_2$did not prevented weight loss when compared to$HgCl_2$-treated group,as well as verapamil.

Table. 1: Effects of Eleusine indica aqueous extract on body weight

Body weight (g)	NaCl 0.9 %+D. W.	$HgCl_2$+D. W.	$HgCl_2$+vera. 0.5 mg/kg	$HgCl_2$+Ext. 100 mg/kg	$HgCl_2$+Ext. 200 mg/kg
Initial	141.50 ± 5.56	147.25 ± 2.21	147.25 ± 2.84	140.75 ± 4.71	142.25 ± 2.93
Final	161.00 ± 3.72	137.80 ± 5.20[a]	142.20 ± 8.26	131.80 ± 7.28[a]	139.20 ± 8.50

Each value represents means ± S.E.M. of 5 rats. [a]$p<0.05$ significantly different compared to normal rats. Vera 0.5 mg/kg: verapamil 0.5 mg/kg, Ext. 100 mg/kg: Extract 100 mg/kg, Ext. 200 mg/kg: Extract 200 mg/kg.

3.2. Effects of *Eleusine indica* aqueous extract on the relative weight of liver and kidney weights:

The liver and kidney relative weights of treated and control groups are shown in Table. 2. Daily administration of $HgCl_2$for 30 days caused a significant increase ($p<0.05$) in the relative weight of liver and kidney by 34.18 % and 38.24 % respectively as compared to control group. The simultaneous administration of the plant extract with $HgCl_2$ prevented the increase in the relative liver weight although non-significant as compared to $HgCl_2$-treated group. However, the plant extract did not affect the increase in kidney relative weight as compared to $HgCl_2$-treated group.

Table. 2: Effect of Eleusine indica aqueous extract on relative liver and kidneys weights

Relative organ weights (%)	NaCl 0.9 %+D. W.	$HgCl_2$+D. W.	$HgCl_2$+vera. 0.5 mg/kg	$HgCl_2$+Ext. 100 mg/kg	$HgCl_2$+Ext. 200 mg/kg
Liver	3.16 ± 0.17	4.24 ± 0.01[a]	3.97 ± 0.44	3.74 ± 0.14	3.93 ± 0.18
Kidneys	0.68 ± 0.05	0.94 ± 0.03[a]	0.99 ± 0.09[a]	1.07 ± 0.05[a]	1.09 ± 0.10[a]

Each value represents means ± S.E.M. of 5 rats. [a]$p<0.05$ significantly different compared to normal rats. Vera 0.5 mg/kg: verapamil 0.5 mg/kg, Ext. 100 mg/kg: Extract 100 mg/kg, Ext. 200 mg/kg: Extract 200 mg/kg.

3.3. Preventive effect of the aqueous extract of *Eleusine indica* on liver function:

The effects of the aqueous extract of *Eleusine indica*on liver function are shown in table 3. Daily administration of $HgCl_2$ for 30 days caused a significant increase ($p<0.05$) in ALT, AST and ALP activities, whereas total proteins and albumin were significantly decreased as compared to the control group. The increase was by 85.71 %, 116.21 %and75.82 % respectively for ALT, AST andALP. Total proteins and albumin were significantly decreased by 62.78 % ($p<0.001$) and 36.36 % ($p<0.05$) respectively. Total bilirubin in $HgCl_2$-treated group was significantly ($P<0.01$) increased by79.42 %as compared to the control group. The plant extract at the dose of 100 mg/kg given simultaneously with $HgCl_2$ prevented theincrease ($p<0.05$)of ALT, AST, ALP and totalbilirubin by 47.09 %, 61.99 %, 45.37 %and 41.11 %respectively, whereas the levels of total proteins and albumin were increased by 105.56 % ($p<0.001$)and 53.27 %($p<0.01$)ascompared to $HgCl_2$-treated rats. The plant extract at the dose of 200 mg/kg administered with $HgCl_2$ prevented the increase of ALT, AST and total bilirubin by 66.71 % ($p<0.01$), 63.56 %($p<0.05$) and 27.96 % ($p<0.05$)respectively, whereas the levels of total proteins and albumin were respectively increased by 66.98 % ($p<0.01$)and 56.08 %($p<0.05$). Verapamil used in the samecondition significantly prevented the changein these parameters.

Table. 3: Preventive effect of the aqueous extract of Eleusine indica on liver function

	Treatments				
Parameters	NaCl 0.9 %+D. W.	$HgCl_2$+D. W.	$HgCl_2$+Vera. 0.5 mg/kg	$HgCl_2$+Ext. 100 mg/kg	$HgCl_2$+Ext. 200 mg/kg
ALAT (U/L)	16.30 ± 2.25	30.27 ± 2.56^{a}	6.69 ± 2.63$^{\gamma}$	16.02 ± 4.38$^{\alpha}$	10.08 ± 3.87$^{\beta}$
ASAT (U/L)	3.64 ± 0.30	7.87 ± 1.56^{a}	2.35 ± 0.46$^{\beta}$	2.99 ± 1.02$^{\alpha}$	2.87 ± 1.00$^{\alpha}$
ALP (U/L)	5.21 ± 0.24	9.16 ± 1.62^{a}	3.93 ± 0.47$^{\beta}$	5.00 ± 0.46$^{\alpha}$	6.55 ± 0.78
Total bilirubin (mg/dL)	128.83 ± 13.80	231.15 ± 23.57^{b}	93.23 ± 23.20$^{\gamma}$	136.13 ± 8.76$^{\alpha}$	166.51 ± 24.60
Total proteins (mg/dL)	8.84 ± 0.60	3.29 ± 0.14^{c}	5.74 ± 0.31$^{\beta}$	6.76 ± 0.19$^{\gamma}$	5.49 ± 0.5$^{\beta}$
Albumin (mg/dL)	2.42 ± 0.15	1.54 ± 0.11^{a}	2.55 ± 0.20$^{\alpha}$	2.36 ± 0.14$^{\alpha}$	2.40 ± 0.30$^{\alpha}$

Each value represents means ± S.E.M. of 5 rats. $^{a}p<0.05$,$^{b}p<0.01$,$^{c}p<0.001$ significantlydifferent compared to normal rats. $^{\alpha}p<0.05$,$^{\beta}p<0.01$, $^{\gamma}p<0.001$, significantly different compared to hepato-nephrotoxic rats. Vera 0.5 mg/kg: verapamil 0.5 mg/kg, Ext. 100 mg/kg: Extract 100 mg/kg, Ext. 200 mg/kg: Extract 200 mg/kg.

3.4. Preventive effect of the aqueous extract of eleusine indica on lipid profile:

The effects of the aqueous extract of*Eleusine indica*on lipid profile were evaluated by the determination in serum levels of total cholesterol, triglycerides, LDL and HDL- cholesterol as shown in Table4.Daily administration of $HgCl_2$for 30 days caused a significant increase ($p<0.001$) in total cholesterol, TG, LDL-cholesterol levels, anda significantly decrease ($p<0.001$) in HDL-cholesterol as compared to the control group. Concomitant administration of the plant extract with $HgCl_2$ significantly prevented these variations. The plant extract at the dose of 100 mg/kg inhibited the increase in thetotal cholesterol, triglycerides and LDL- cholesterol by 57.00 % ($p<0.01$), 89.14 % ($p<0.001$) and 59.34 % ($p<0.01$), whereas the inhibition ($p<0.001$) of these parameters was by 86.17 %, 62.54 %, 91.60 % at the dose of 200 mg/kg as compared to $HgCl_2$-treated group. The plant extract at the dose of 100 and 200 mg/kg prevented the decrease in HDL-c respectively by 239.02 % ($p<0.01$) and 343.93 % ($p<0.001$) as compared to $HgCl_2$-treated group. Verapamil used in the same condition significantly prevented the change in these parameters. Each value represents means ± S.E.M. of 5 rats. $^{c}p<0.001$ significantly different compared to normal rats.

$^{\beta}p<0.01$, $^{\gamma}p<0.001$, significantly different compared to hepato- nephrotoxic rats. Vera 0.5 mg/kg: verapamil 0.5 mg/kg, Ext. 100 mg/kg: Extract 100 mg/kg, Ext. 200 mg/kg: Extract 200 mg/kg.

3.5. Preventive effect of the aqueous extract of *Eleusine indica* on kidney function:

Table. 5 illustrates the effects of the aqueous extract of *Eleusine indica* on some parametersof kidney function. Concomitant administration of distilled water and$HgCl_2$caused a significant increase ($p<0.001$) in serum levels of creatinine, urea, uric acid and K^+ respectively by 70.69 %, 114.92 %, 69.30 % and 313.13 %, while $HgCl_2$induced significant reduction ($p<0.05$) in serum level of Na^+ by 64.74 % as compared to the control group. The administration of the plant extract with $HgCl_2$prevented the increase in serumconcentration of creatinine (39.63 %; $p<0.001$), urea (37.25 %, $p<0.01$), uric acid (49.35 %, $p<0.01$), K^+ (67.09 %, $p<0.01$) and a decrease in serum Na^+ (217.11 %, $p<0.05$) at the dose of 100 mg/kg as compared to $HgCl_2$-treated group. At the dose of 200 mg/kg, the increase of serum creatinine, urea, uric acid, and K^+ was respectively by 39.60 %($p<0.001$), 77.54 % ($p<0.001$), 41.89 % ($p<0.05$), 68.43 % ($p<0.01$) while the increasein Na^+ was by 232.58 % ($p<0.05$). Verapamil administered in the same condition significantly prevented the change in these parameters. *Table. 4: Preventive effect of the aqueous extract of Eleusine indica on lipid profile*

	Treatments				
Parameters	NaCl 0.9 %+D. W.	$HgCl_2$+D. W.	$HgCl_2$+Vera. 0.5 mg/kg	$HgCl_2$+Ext. 100 mg/kg	$HgCl_2$+Ext. 200 mg/kg
Total cholesterol (mg/dL)	41.64 ± 7.89	188.41 ± 16.7^{c}	75.38 ± 24.57$^{\beta}$	81.03 ± 23.58$^{\beta}$	22.97 ± 3.95$^{\gamma}$
Triglycerides (mg/dL)	50.00 ± 6.59	169.88 ± 35.2^{c}	44.25 ± 9.32$^{\gamma}$	20.96 ± 7.39 $^{\gamma}$	72.36 ± 10.13$^{\gamma}$
HDL-c (mg/dL)	20.42 ± 2.53	5.34 ± 1.59^{c}	17.80 ± 2.43$^{\beta}$	18.11 ± 3.09$^{\beta}$	23.71 ± 1.77$^{\gamma}$

LDL-c (mg/dL)	11.22 ± 6.79	149.09 ± 10.9^{c}	48.74 ± 25.46$^{\beta}$	58.72 ± 23.97$^{\beta}$	15.21 ± 5.84$^{\gamma}$

Table. 5: Preventive effects of the aqueous extract of Eleusine indica on kidney function

	Treatments				
Parameters	NaCl 0.9 %	$HgCl_2$ 0.02 mg/kg	$HgCl_2$+Vera. 0.5 mg/kg	$HgCl_2$+Ext. 100 mg/kg	$HgCl_2$+Ext. 200 mg/kg
Creatinine (mg/dL)	0.62±0.05	2.27±0.49^{c}	0.79±0.08$^{\beta}$	0.69±0.04$^{\gamma}$	0.69±0.03$^{\gamma}$
Urea (mg/dL)	9.72±0.83	20.90±1.22^{c}	9.38±0.88$^{\gamma}$	13.11±0.50$^{\beta}$	4.69±1.93$^{\gamma}$
Uric acid (mg/dL)	3.69±0.60	6.26±0.84^{c}	3.31±0.13$^{\beta}$	3.17±0.11$^{\beta}$	3.64±0.53$^{\alpha}$
Na^+ (mg/dL)	114.02±15.3	40.20±14.12^{a}	149.46±14.46$^{\beta}$	127.48±21.1$^{\alpha}$	133.70±17.8$^{\alpha}$
K^+ (mg/dL)	7.15±2.27	29.55±3.64^{c}	4.15±0.7$^{\gamma}$	9.72±3.98$^{\beta}$	9.33±1.13$^{\beta}$

Each value represents means ± S.E.M. of 5 rats. $^{a}p<0.05$,$^{c}p<0.001$ significantly different compared to normal rats. $^{\alpha}p<0.05$, $^{\beta}p<0.01$, $^{\gamma}p<0.001$, significantly different compared to hepato-nephrotoxic rats. Vera 0.5 mg/kg: verapamil 0.5 mg/kg, Ext. 100 mg/kg: Extract 100 mg/kg, Ext.200 mg/kg: Extract 200 mg/kg.

3.6. Preventive effects of *Eleusine indica* aqueous extract on some markers of oxidative stress:

The effects of *Eleusine indica* aqueous extracton some markers of oxidative stress are shown in Fig. 1. Treatment with$HgCl_2$induced a significant increase($p<0.001$) in liver and kidney MDA concentration respectively by 606.79 % and 383.42 %as compared to control group (Fig 1A). The extract administered with $HgCl_2$prevented the increase ($p<0.001$) in MDA concentration in the liver (85.58 %) andin the kidney (77.40 %) at the dose of 100 mg/kg. At the dose of 200 mg/kg, it was observed a decrease ($p<0.001$) in MDA concentration by 57.86 % and by 70.40 % respectively in the liver and kidney. The treatment with $HgCl_2$during 30 days induced significant decrease ($p<0.05$) in catalase activity by 53.54 % and by 54.08 % respectively in the liver and kidney as compared to NaCl group (Fig 1B). Concomitant administration of $HgCl_2$with plant extract significantly ($p<0.05$) prevented the decrease in catalase activity by 133.57 % and 120.56

% in the liver and kidney at the dose of 200 mg/kg. Treatment with$HgCl_2$induced a significant decrease in liver (50.00 %, $p<0.05$) and kidney GSH concentration (66.67 %, $p<0.01$) as compared to control group (Fig 1C). The extract administered with $HgCl_2$prevented the decrease in GSH concentration by 100.00 % ($p<0.05$) in the liver and by 200.00 %($p<0.001$) in the kidney at the dose of 100 mg/kg. At the dose of 200 mg/kg, it was observed an increase in GSH concentration by 100.00 % ($p<0.001$) in the kidney. The administration of$HgCl_2$during 30 days induced significant decrease ($p<0.001$) in SOD activity respectively in the liver (3.52%) and the kidney (4.26 %) as compared to NaCl group (Fig 1D). Concomitant administration of $HgCl_2$with plant extractsignificantly ($p<0.001$) prevented the decreasein SOD activity by 3.88 % and 5.56 %, and by 4.13 % and 6.10 % in the liver and kidney respectively at all dose.

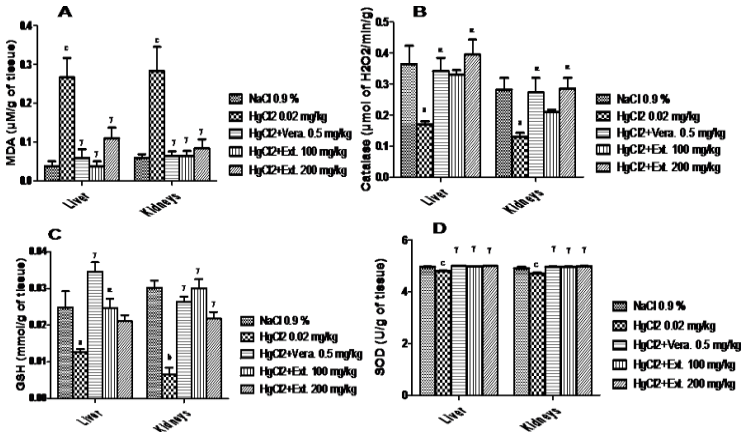

Figure. 1:

Fig 1: Effects of *E. indica* aqueous extract on some markers of oxidative stress in Hg-induced hepato-nephrotoxicity. Each bar represents means ± S.E.M. of 5 rats; $^{a}p<0.05$,$^{b}p<0.01$,$^{c}p<0.001$ significantly different compared to normal rats (NaCl 0.9 %). $^{\alpha}p<0.05$, $^{\gamma}P<0.001$ significantly different compared to hepato-nephrotoxic rats ($HgCl_2$ 0.02 mg/kg). Ext. 100 mg/kg: Extract 100 mg/kg, Ext. 200 mg/kg: Extract 200 mg/kg, Vera. 0.5

mg/kg: verapamil 0.5 mg/kg

4. Discussion:

This study aimed to evaluate the preventive effects of aqueous extract of the whole plantof *Eleusine indica* against mercuric chloride- induced hepato-nephrotoxicity in rat. Mercuric ion, one of the strongest thiol- binding agent, increases the intracellular levels of reactive oxygen species and induces oxidative stress resulting in liver and kidneys damages.

The administration of mercury chloride ($HgCl_2$) for 30 daysto rats induced a significant reduction in body weight, contrary to a significant increase in liver and kidneys relative weights as compared to control. The decrease in body weight observed could be due to abnormal growth resulting from a decrease of food and water intake. Grossman Berthound *et al.* and in their works have found that mercury reduce themean food intake and body weight and causedlesions in the brain areas involved in the regulation of food intake resulting inhypophagia.

The increase in liver and kidneys relative weight could be due to inflammatoryresponse. It is well known that the reductionor increase in the weight of internal organs is an indication of toxicity after exposure to a toxic substance [23]. In fact, any abnormal stimulus to the kidney or liver triggers an inflammatory response resulting in theincrease of these organs. Therefore the increase in the weight of liver and kidneys observed in the present study indicates theinflammatory reaction following mercury chloride administration. Co-administration of $HgCl_2$with*Eleusine indica* aqueous extract at the doses of 100 and 200 mg/kg to animals slightly reduced the hypertrophy of liver, while slightly increased that of the kidneys, but not in significant manner. These results suggested that the plant extract could prevent the inflammatory response of liver while it was not able to prevent that of the kidneys. Our results also shown that the loss in body weight was slightly but non-significantlyreduced in the animals treated with the plant extract as compared to negative controls, thus indicating that the extract may improve water and food intake by preserving brain areas involved in the regulation of food intake, thus hypophagia. Phytochemical analyses of *Eleusine indica* aqueous extract revealed the presence of saponins which are known to possess anti-inflammatory properties. Thus,such compounds may explain in part the slight inhibition observed on the liver hypertrophy.

In the present study, administration of $HgCl_2$to rats for 30 days also significantly increased activities of transaminases (ALT and AST) and alkaline phosphatase (ALP), as well as the level of total bilirubin. In contrast the serum levels of total proteins and albumin were significantly reduced as compared to control rats. Serum ALT, AST, ALP and total bilirubin are recognized as conventional markers of hepatotoxicity, and their levels in blood reflect the alterations of liver. Our results therefore indicate that$HgCl_2$probablyinduced hepato-cellularnecrosis or membrane damage resulting to therelease of these enzymes into the blood circulation. Significant reduction in the serum level of total proteins and albumin maybe attributed to a decline in protein synthesis by hepatic cells reflecting the hepaticdysfunction Co-administration of $HgCl_2$with *Eleusine indica* aqueous extract at the doses of 100 and 200 mg/kg for 30 days torats significantly inhibited the

increase of transaminases (ALT and AST) and alkaline phosphatase activities, as well as the level of total bilirubin, whereas the serum levels oftotal proteins and albumin were increased. These results of the aqueous extract of*Eleusine indica* may be due to its ability to prevent hepato-cellular necrosis or membrane damages. These effects could be attributed to compounds such as glycosides and phenolsdetected in the extract, which act by stimulating the synthesis of the genes responsible of cellular regeneration

Daily $HgCl_2$ administration caused a significant increase in serum levels of total cholesterol, triglycerides and LDL-C, with a concomitant decrease of HDL-C in $HgCl_2$-treated group as compared to control rats. A report mentioned that mercury species promote cardiovascular disorders viametabolic changes of cholesterol and triglycerides, suggesting that these parameters may consequently be involved in the increase in $HgCl_2$-induced cardiovascular risks.Indeed, $HgCl_2$ injection is thought to reduce the activity of the lipoprotein lipase and triglyceride lipase enzymes, thus resulting in the decreased uptake of triglycerides from serum causing its accumulation. Inaddition, the elevation of cholesterol level observed may be due to the increased in the activity of the enzyme β- hydroxymethylglutaryl CoA (HMGCoA)which catalyzes the rate limiting step in cholesterol biosynthesis leading to increased cholesterol synthesis in tissues and excess leaking out of cholesterol into the blood. The decrease of HDL may be due to the decrease of cholesterol ester transfer protein (CETP)activity which transfers TG from VLDL to HDL. HDL charged with TG is quickly hydrolyzed and due to the fact of their higher catabolism, HDL blood level decreases and that of LDL increases [31].The increase of total cholesterol and triglycerides, and the decreaseof HDL-cholesterol observed in this study may be respectively due to the increased inthe activity of the HMGCoA, the reduction of the activity of the lipoprotein lipase and triglyceride lipase enzymes and the decreaseof CETP activity. Co-administration of $HgCl_2$with the aqueous extract of *Eleusine indica* at the doses of 100 and 200 mg/kg to rats improved the lipid profile resulting to the decrease of total cholesterol, triglycerides andLDL-cholesterol, and to the increase of HDL- cholesterol. These results suggest that this extract may increase the activity of thelipoprotein lipase and triglyceride lipase enzymes, and reduce the activity of the enzyme HMGCoA, allowing restraining fatstorage and dyslipidemia. Phytochemical studies revealed the presence of phenols and alkaloids compounds whose hypolipidemic activities were shown. Indeed, phenols bind tocholesterol in the digestive tract in order to prevent their intestinal reabsorption and to increase their elimination. Alkaloidsstimulate hepatic catabolism of LDL to HDL and reduction in the level of LDL in favour ofHDL leading to the reduction in cholesterol. Our results showed

that $HgCl_2$ injection in rats increased the concentration of creatinine, urea and uric acid as compared to control.These increases indicate nephrotoxicity. It is well known that mercury accumulates more in renal epithelium. Creatinine derives from endogenous sources, by tissue creatine breakdown and its clearance enables a quite good estimation of the glomerular filtration rate. A significant increase in creatinine level could possibly be a result of accumulation of mercury chloride in the proximal tubular cells which causes the inhibition of lysosomal phospholipidosis, inducing proximal tubular necrosis. Urea is the nitrogen containing end product of proteincatabolism. The concentration of urea iselevated when glomerular filtration rate is markedly decreased in renal failure. Moreover, urea concentration begins to rise only after parenchymal tissue damage. Thepossible reason behind the serum urea accumulation may be an increase rate of serum urea production than the clearance rate. Uric acid is the end product of purine metabolism; hyperuricemia is associated with impaired renal function. High levels of serum creatinine, urea and uric acid can be used as a rough index of the glomerular filtration rate and indicates several disturbances in kidney [41].Serum concentrations of creatinine, urea and uric acid are three of the traditional screening indices for kidney functions and renal structural integrity. Elevation in creatinine, urea and uric acid might be due to kidney tubulesdamages. These results observed in our work are undoubtedly related to acute and persistent renal injuries, thus confirming that the kidneys are very sensitive to mercury exposition.

Eleusine indica aqueous extract (100 and 200 mg/kg) counteracted these effects so that, serum concentrations of creatinine, urea and uric acid were decreased in rats receiving both $HgCl_2$ injection and plant extract. The plant extract prevented creatinine, urea and uric acid increases, suggesting that this extract might interfere with mechanisms of $HgCl_2$-induced injuries in kidney. The protective roleof *Eleusine indica* aqueous extract may beexplained by the capacity of this extract to prevent proximal tubular necrosis, parenchymal tissue damage andimpairment ofrenal function. Our results showed a significant increase in serum levelofpotassium, with a significant depletion in sodium level in $HgCl_2$-treated rats ascompared to controls. It was demonstrated that the treatment of rats with $HgCl_2$ significantly enhanced serum levels of K^+, and significantly decreased the level of Na^+. $HgCl_2$ induces generation of free radicals species, resulting in oxidative cell damages, which can cause cell membrane damages which in turn inactivated membrane Na^+–K^+ ATPase pump, thereby allows entry of Ca^{+2} into the cell. The sustained increase in intracellular Ca^{+2} leads to generation of free radicals, which in turn cause inhibition of Na^+–K^+ ATPase pump and impair antioxidantstatus. Therefore the decrease of Na^+ and the increase of K^+ observed in this

study are probably due to the generation of free radicalsand the inhibition of Na^+–K^+ ATPase pump. The concomitant administration of $HgCl_2$and the aqueous extractof *Eleusine indica* has decreased the level of K^+ and increased that ofNa^+, suggesting that this extract might interfere with the generation of free radicals and the inhibition of Na^+–K^+ ATPase pump.Indeed, cardiac glycosides and phenols present in our extract can act by stimulating the synthesis of the genes responsible of cellular regeneration of renal tissue (Rajendran *et al*., 2009). From the present results, the level of GSH,and the activities of catalase and SOD were significantly decreased in the kidney and livertissues of $HgCl_2$-treated rats as compared to control group, which indicated that mercury has caused severe oxidative stress.$HgCl_2$-induced hepaticand renal oxidant stress were evident and indicated by significant elevationsin lipid peroxidation (MDA) in these tissues of $HgCl_2$-treated rats as compared to controls. Toxicity of mercury is associated with superoxide and peroxide radical generation,as well as glutathione reduction. In fact, it can be hypothesized that oxidative stress may be one of the contributing factors for Hg- induced organs dysfunction. Increasedreactive oxygen species (ROS) were reported in previous studies during $HgCl_2$ exposure. Subsequently, ROS attacks almost all cellcomponents including membrane lipids. Therefore the increase of MDA and the decrease of SOD, catalase and GSH may be the consequence of the action of ROS in liver and kidney tissues causin gdestruction of cell membranes. Co-administration of$HgCl_2$ with *Eleusine indica* aqueous extract prevented the increase of MDA, and the decrease of SOD, catalase and GSH levels induced by $HgCl_2$, suggesting that tis extract may prevent the generation of free reactive oxygen species andthe destruction of cell membranes. Thus *Eleusine indica* aqueous extract may have antioxidant properties. These properties may be related to the presence in this extract of compounds like flavonoids, tannins, alkaloïds which are able to scavenge free radical and protect the cell membrane from destruction.

5. Conclusion:

The administration of $HgCl_2$ for 30 days results in the decrease in body weight, total proteins and albumin levels, and in theincrease in relative organ weights, ALT, AST,ALP activities and total bilirubin. Hypercholesterolemia and hypertriglyceridemia were also observed in $HgCl_2$-treated group. Renal parameters have shown an increase in concentration of creatinine, urea, uric acid and K+ with a decrease of Na^+ concentration. Antioxidant status has shown an increase of MDA in liver and kidney, with a decrease of catalase, SOD and GSH in these organs. However concomitant

administration of $HgCl_2$ with the plant extract prevented body weight loss, improved hepatic parameters, lipid profile, renal parameters and antioxidant status. Thus, these results suggest that *Eleusine indica* aqueous extract exhibited hepato- nephroprotective effects. These activities might be related to its antioxidant potential and supports the traditional use of the whole plant of *Eleusine indica* to manage hepaticand renal disorders.

6. References

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