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HEPATO-NEPHROPROTECTIVE EFFECTS OF AQUEOUS EXTRACT OF *ELEUSINE INDICA* (LINN) GAERTN. (POACEAE) AGAINST GENTAMICIN-INDUCED HEPATIC AND RENAL TOXICITY IN RAT

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ABSTRACT

Eleusine indica is traditionally used in Cameroonian folk medicine to treat several diseases including renal and hepatic disorders. The present study was to evaluate hepato-nephroprotective effects of *Eleusine indica* aqueous extract against gentamicin-induced hepatic and renal toxicity in rat. Rats were divided into control group and experimental group. Control group was pretreated intraperitoneally (ip) with saline 0.9 % (1 mL/100 g) during 10 days, while experimental group was pretreated by administration of gentamicin (ip route) at the dose of 100 mg/kg during 10 days. Animals of experimental group were then subdivided into four groups treated during twenty consecutive days as follow: control group: rats were treated with 0.9 % NaCl ip, negative group: rats were receiving gentamicin (100 mg/kg), positive group: rats were receiving gentamicin and aspirin (80 mg/kg), extract group I and II: rats were receiving gentamicin and *Eleusine indica* aqueous extract (100 and 200 mg/kg). The effects of *Eleusine indica* aqueous extract were evaluated after 30 days on liver, kidney and antioxidant parameters by colorimetric method. Histopathological examination of rat liver and kidney was done. Gentamicin induced hepatotoxicity by significant elevation in serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), serum levels of total bilirubin, total cholesterol (Chol), triglycerides (TG) and LDL, with significant reduction of HDL as compared to control rats. Nephrotoxicity was evidenced by elevation in serum levels of creatinine, urea, uric acid and potassium (K^+), with significant reduction of sodium (Na^+) as compared to control rats. MDA and nitrites levels were significantly increased in gentamicin-injected group, while catalase and SOD activities and GSH level were significantly decreased as compared to control rats. The histopathologic studies of liver of rats injected with gentamicin revealed leucocytic cells infiltration, fibrosis and dilatation of sinusoidal capillaries, while kidneys showed fibrosis, leucocytic cells infiltration. *Eleusine indica* aqueous extract has corrected various modifications of biochemical and oxidative markers evaluated. This study shows that *Eleusine indica* aqueous extract can correct gentamicin-induced hepato-nephrotoxicity in rats and attenuates liver and kidneys damages caused by gentamicin injection and oxidative stress.

KEYWORDS: *Eleusine indica*, gentamicin, hepatotoxicity, nephrotoxicity, oxidative markers.

INTRODUCTION

Aminoglycosides such as gentamicin, are natural or semisynthetic antibiotics with a heterocyclic structure formed by two or more amino sugars linked by glycoside bounds to an aminocyclitol ring, the most widely used drug in this category is gentamicin (GM).[1] However, the usefulness of gentamicin is limited by the development of nephrotoxicity and hepatotoxicity. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis (mainly in proximal tubules), basal membrane disruption, mesangial cell contraction, proliferation and apoptosis, thus indicated by decreases in glomerular filtration and alteration in intraglomerular dynamics.[2] These mechanisms can leed to the generation of hypertension witch is a major complication of renal failure.[3] The pathogenesis of gentamicin-induced nephrotoxicity involves multiple pathways, including oxidative stress, inflammation, reduced renal blood flow, and increased nitric oxide (NO) level.[4-5] Recent evidence showed that reactive oxygen species (ROS) play a pivotal role in gentamicin-mediated nephrotoxicity, it induced impairment of renal function through liberation of ROS

in rats.[6] Abnormal production of ROS directly damages some macromolecules and induces necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation, and DNA damage. Accordingly, the administration of several compounds with antioxidant activity has been successfully used to prevent or ameliorate GM-induced nephrotoxicity.[7]

Eleusine indica (*E. indica*) is a native plant of the tropics and subtropical regions, belonging to the Poaceae family. The whole plant, especially the root, is used in traditional medicine as a diuretic, anti-helminthic, febrifuge and for treating cough or other ailments.[8] The decoction of the whole plant is consumed as anti-helminthic and febrifuge treatments. The seed is sometimes used as famine food and also used in the treatment of liver complaints.[9] Studies have also found that C-glycosylflavones from *E. indica* have anti-inflammatory effects on lipopolysaccharide-induced lung airway inflammation in mice. The infusion of aerial parts of *E. indica* is used in Brazil against airway inflammatory processes, such as pneumonia.[10] The aqueous extract of *Eleusine indica* contains primary as well as secondary metabolites such as alkaloïds, saponins, flavonoïds, cardiac glycosides, phenols and tannins.[11] Information provided by traditional healers in Center Region of Cameroon indicates that the whole plant of *Eleusine indica* is used in the management of hepatic and renal problems. Previous studies shown that aqueous extract of the whole plant of *E. indica* prevented L-NAME-induced nephrotoxicity.[11] The present study was designed to evaluate the hepato-nephroprotective effects of aqueous extract of *E. indica* against gentamicin-induced hepatic and renal toxicity in young rats.

MATERIALS AND METHODS

Harvest of the whole plant and preparation of extract

The whole plant of *Eleusine indica* was harvested from Ngoa-Ekelle (Yaoundé-Cameroon), in January 2015, and authenticated by mister NGANSOP TCHATCHOUANG Eric at the National Herbarium, Yaoundé, where a specimen has been deposited in comparison to voucher number N° 8356 SRF/CAM (YA). The whole fresh plant was washed throughly tap water, air dried in the shade at room temperature and reduced in powder. The powder (300 g) was boiled in 5 L of tap water during 20 minutes according to the traditional healer's instructions. The mixture was filtered with Whatman N° 3 filter paper. The solution obtained was evaporated at 45°C in drying cupboard and gave 15.8 g of the aqueous extract (yield 5.27 %).

Animals

Eight week old male *Wistar* rats, weighting between 130 and 160 g were used. Animals were raised in the Animal House of the Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1 (Cameroon), in plastic cages, under natural luminosity cycle and temperature, with free access to normal laboratory rat food and tap water. All the procedures in this study followed the principles of laboratory animal use and care, and were approved by the Cameroon National Ethical Committee (authorization number FW-IRB00001954).

Animals grouping and treatments

Twenty five rats were divided into control group (I) (n=5) and experimental group (n=20). Control group was pretreated intraperitonealy (ip) with NaCl (0.9 %), while experimental group received gentamicin 100 mg/kg, ip for 10 days to induced nephrotoxicity. Animals of experimental group were then subdivided into four groups of five animals each treated during twenty consecutive days as follow: group I (control) was treated with 0.9 % NaCl ip simultaneously with distilled water (orally at the dose of 10 mL/kg) ; Group II (gentamicin-treated group) was given a single injection of gentamicin (ip) at the dose of 100 mg/kg simultaneously with distilled water (orally at the dose of 10 mL/kg) ; Group III (Asp + gentamicin-treated group) was given a single injection (ip) of gentamicin at the dose of 100 mg/kg simultaneously with aspirin solution (orally at the dose of 80 mg/kg) ; Group IV and V (Ext.+gentamicin-treated group) were given a single injection (ip) of gentamicin (100 mg/kg) simultaneously with *E. indica* aqueous extract orally at respective doses of 100 and 200 mg/kg.

Hemodynamic parameters recording

At the end of the experimental period, arterial blood pressure and heart rate of all rats were recorded. The rats were anesthetized using urethane (1.5 g/kg, ip). The trachea was exposed and cannulated to facilitate spontaneous breathing. The arterial blood pressure was recorded by using the method of Van Viet *et al*.[12]

Assessment of liver and kidney functions

Blood samples were immediately collected after hemodynamic parameters measurement in dried tubes and the serum was obtained by centrifugation at 3000 rpm during 15 min at 4°C for the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, total cholesterol (Chol), triglycerides (TG), HDL-Cholesterol (HDL-c), creatinine, urea, uric acid, sodium (Na^+) and potassium (K^+) levels using commercial diagnostic kits (Fortress from UK for total bilirubin, creatinine and urea, and Inmesco from Germany for ALT, AST, CHO and HDL, SGMitalia from Italia for TG and uric acid, Spectrum from Switzerland for Na^+ and K^+). The level of LDL-Cholesterol (LDL-c) was determined using the formula: LDL-Cholesterol (mg/dL) = CHO - (TG/5) – HDL. [13]

Assessment of hepato-renal oxidative stress

After blood collection, the abdominal cavity was opened. Liver and kidneys were collected and homogenized with Tris-HCl 50 mM buffer solution to make a 20 % homogenate. Malondialdehyde (MDA) was determined using the procedure of Wilbur *et al.*[14] Catalase activity was determined according to Sinha [15], whereas reduced glutathione (GSH) was determined using the method

described by Ellman [16]. Superoxide dismutase (SOD) activity was determined using the method described by Misra and Fridovish [17]. The nitrites levels in the tissues were determined using the Griess reagent method.[18] A part of liver and kidney was cut and was fixed in 10% formalin solution for histological evaluation using Van Geison coloration.

Statistical analysis

Data were expressed as mean ± standard error of mean (S.E.M). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. A value of $P<0.05$ was considered statistically significant. All analyses were performed using Graphpad prism software 5.03 version.

RESULTS AND DISCUSSION

Effects of *E. indica* whole plant aqueous extract on relative organs weight

The variations of liver and kidneys weights are shown in Table 1. Kidneys relative weights were significantly ($p<0.05$) increased by 24.24 % in gentamicin treated group as compared to control group.

Table 1: **Effects of treatment with *E. indica* on relative organs weight.**

	Relative organs weight (%)	
	Liver	**Kidneys**
NaCl 0.9 %	3.40±0.04	0.66±0.02
Genta. 100 mg/kg	3.26±0.09	0.82±0.05*
Genta.+Asp. 80 mg/kg	3.22±0.15	0.78±0.03
Genta.+Ext. 100 mg/kg	3.26±0.07	0.76±0.04
Genta.+Ext. 200 mg/kg	3.21±0.05	0.76±0.03

Each value represents means ± S.E.M. of 5 rats ; *$p<0.05$ significantly different as compared to normal rats (NaCL 0.9 %). NaCl 0.9 %: sodium chloride 0.9 %, Genta 100 mg/kg: gentamicin 100 mg/kg, Genta.+Asp. 80 mg/kg: gentamicin+aspirin 80 mg/kg, Genta.+Ext. 100 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (100 mg/kg), Genta.+Ext. 200 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (200 mg/kg).

Effects of treatment with *E. indica* whole plant aqueous extract on the hepatic biomarkers

Daily administration of gentamicin for 30 days significantly increased ALT ($p<0.05$), AST ($p<0.001$) and total bilirubin ($p<0.001$) as compared to control group (Table 2). Administration of *E. indica* aqueous extract at the dose of 200 mg/kg significantly decreased activity of ALAT by 50.69 % ($p<0.01$) and ASAT by 40.00 % ($p<0.05$) as compared to gentamicin-treated group. At both doses (100 and 200 mg/kg), *E. indica* aqueous extract significantly decreased ($p<0.01$) total bilirubin level by 47.91 % and 44.89 % as compared to negative group.

Table 2: Effects of treatment with *E. indica* whole plant aqueous extract on the hepatic biomarkers.

	Treatments				
Parameters	**NaCl 0.9 %**	**Genta. 100 mg/kg**	**Genta.+Asp. 80 mg/kg**	**Genta.+Ext. 100 mg/kg**	**Genta.+Ext. 200 mg/kg**
ALT (U/L)	15.10±0.92	26.24±2.93*	14.38±0.89$	20.22±3.09	12.94±2.68$$
AST (U/L)	10.76±0.00	53.92±2.33***	26.96±2.20$$	37.74±2.20	32.35±4.40$
Total bilirubin (mg/dL)	59.81±10.89	143.91±16.10***	66.03±10.22$$	74.96±3.91$$	79.31±13.03$$

Each value represents means ± S.E.M. of 5 rats; *$p<0.05$, ***$p<0.001$ significantly different as compared to normal rats (NaCL 0.9 %). $$p<0.05$, $$$p<0.01$ significantly different as compared to gentamicin group. NaCl 0.9 %: sodium chloride 0.9 %, Genta 100 mg/kg: gentamicin 100 mg/kg, Genta.+Asp. 80 mg/kg: gentamicin+aspirin 80 mg/kg, Genta.+Ext. 100 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (100 mg/kg), Genta.+Ext. 200 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (200 mg/kg) ; ALT: alanine aminotransferase ; AST: aspartate aminotransferase.

Effects of treatment with *E. indica* whole plant aqueous extract on lipid profile

Gentamicin significantly increased total cholesterol ($p<0.001$), triglycerides ($p<0.001$), LDL-c ($p<0.01$) and atherogene index ($p<0.001$), while HDL-c was significantly decreased ($p<0.001$) as compared to control group (Table 3). Administration of *E. indica* aqueous extract at the doses of 100 and 200 mg/kg significantly decreased total cholesterol by 62.74 % ($p<0.001$) and 52.62 % ($p<0.001$), triglycerides by 39.63 % ($p<0.01$) and 34.63 % ($p<0.05$), LDL-c by 56.99 % ($p<0.05$) at the dose of 200 mg/kg, and atherogene index at both doses by 76.045 % ($p<0.001$) and 73.89 % ($p<0.001$) as compared to negative group. *E. indica* aqueous extract at both doses (100 and 200 mg/kg) significantly increased

HDL-c level by 27.09 % ($p<0.05$) and 40.69 % ($p<0.001$) as compared to negative group.

Table 3: Effects of treatment with *E. indica* whole plant aqueous extract on lipid profile.

	Treatments				
Parameters	**NaCl 0.9 %**	**Genta. 100 mg/kg**	**Genta.+Asp. 80 mg/kg**	**Genta.+Ext. 100 mg/kg**	**Genta.+Ext. 200 mg/kg**
Chol (mg/dL)	51.65±6.89	$87.04±5.75^{***}$	$44.89±1.79^{\$\$\$}$	$32.43±0.82^{\$\$\$}$	$41.24±3.10^{\$\$\$}$
TG (mg/dL)	85.62±5.85	$241.25±24.73^{***}$	$160.37±15.56^{\$}$	$145.63±18.75^{\$\$}$	$156.88±13.74^{\$}$
HDL-c(mg/dL)	17.78±0.63	$11.92±0.72^{***}$	$15.62±0.75^{\$\$}$	$15.15±0.21^{\$}$	$16.77±0.76^{\$\$\$}$
LDL-c(mg/dL)	8.24±2.21	$26.86±4.99^{**}$	$5.53±0.08^{\$\$}$	14.83±2.36	$11.55±4.99^{\$}$
Atherogene index	2.96±0.45	$7.43±0.71^{***}$	$2.89±0.14^{\$\$\$}$	$1.78±0.07^{\$\$\$}$	$1.94±0.11^{\$\$\$}$

Each value represents means ± S.E.M. of 5 rats; $^{**}p<0.01$,$^{***}p<0.001$ significantly different as compared to normal rats (NaCL 0.9 %). $^{\$}p<0.05$, $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$significantly different as compared to gentamicin group. NaCl 0.9 %: sodium chloride 0.9 %, Genta 100 mg/kg: gentamicin 100 mg/kg, Genta.+Asp. 80 mg/kg: gentamicin+aspirin 80 mg/kg, Genta.+Ext. 100 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (100 mg/kg), Genta.+Ext. 200 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (200 mg/kg) ; Chol: total cholesterol ; TG: triglycerides; HDL-c: HDL-cholesterol ; LDL-c: LDL-cholesterol.

Effects of *E. indica* aqueous extract on the level of renal biomarkers

The effects of *E. indica* aqueous extract on kidney function were evaluated by the determination of creatinine, urea, uric acid, Na^+ and K^+ levels in serum as shown in Table 4. Gentamicin-treated rats induced a significant increase in serum levels of creatinine ($p<0.01$) and uric acid (30.00 %, $p<0.05$) as compared to control group. Urea level were significantly increased ($p<0.001$) in gentamicin-treated group as compared to control group. Serum level of Na^+ in gentamicin-treated group was significantly decreased ($p<0.001$) by 90.99 %, while serum level of K^+ was significantly increased ($p<0.001$) as compared to NaCl 0.9% group. Administration of *E. indica* aqueous extract (100 mg/kg) significantly decreased serum level of creatinine by 58.83 % ($p<0.01$) and urea serum level ($p<0.05$) by 49.64 % as compared to the negative group. *E. indica* aqueous extract at the dose of 200 mg/kg significantly decreased ($p<0.01$) creatinine and uric acid level by 58.82 % and by 25.27 % as compared to the negative group. The extract (100 and 200 mg/kg) prevented ($p<0.05$) the decrease in serum level of Na^+, and significantly prevented ($p<0.01$) the increase in serum level of K^+ by 55.07 % and 61.86 % as compared to negative group.

Table 4: Effects of *E. indica* on the level of renal biomarkers.

	Treatments				
Parameters	**NaCl 0.9 %**	**Genta. 100 mg/kg**	**Genta.+Asp. 80 mg/kg**	**Genta.+Ext. 100 mg/kg**	**Genta.+Ext. 200 mg/kg**
Creatinine (mg/dL)	1.30±0.33	$3.40±0.66^{**}$	$0.50±0.00^{\$\$\$}$	$1.40±0.37^{\$\$}$	$1.40±0.37^{\$\$}$
Urea (mg/dL)	5.83±3.74	$27.88±1.10^{***}$	$8.10±1.96^{\$\$}$	$14.04±3.54^{\$}$	15.49±3.17
Uric acid (mg/dL)	1.40±0.08	$1.82±0.08^{*}$	$1.37±0.07^{\$\$}$	1.57±0.08	$1.36±0.03^{\$\$}$
Na^+ (mmol/L)	171.20±23.70	$15.43±9.51^{***}$	$209.13±31.67^{\$\$\$}$	$135.58±33.73^{\$}$	$148.13±22.69^{\$}$
K^+ (mmol/L)	3.28±0.23	$14.29±1.56^{***}$	$5.07±1.40^{\$\$}$	$6.42±1.90^{\$\$}$	$5.45±0.83^{\$\$}$

Each value represents means ± S.E.M. of 5 rats ; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different as compared to normal rats (NaCL 0.9 %). $^{\$}p<0.05$, $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$ significantly different as compared to hepato-nephrotoxic rats (Gentamicin). NaCl 0.9 %: sodium chloride 0.9 %, Genta 100 mg/kg: gentamicin 100 mg/kg, Genta.+Asp. 80 mg/kg: gentamicin+aspirin 80 mg/kg, Genta.+Ext. 100 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (100 mg/kg), Genta.+Ext. 200 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (200 mg/kg) ; Na^+: sodium ; K^+: potassium.

Effects of *E. indica* aqueous extract on some markers of oxidative stress

Figure 1 shows effects of *E. indica* aqueous extract on tissue SOD and catalase activities, MDA, GSH and Nitrites levels. Gentamicin administration induced a significant increase ($p<0.001$) in liver and kidney MDA concentration as compared to control group (Fig 1A). The extract administered with gentamicin prevented the increase ($p<0.001$) in MDA concentration in the liver (44.83 %) and in the kidney (73.08 %) 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 51.71 % and by 59.69 % respectively in the liver and kidney. The

administration of gentamicin during 30 days induced significant decrease in SOD activity in the kidney (15.66 %, p<0.05) as compared to NaCl group (Fig 1B). Concomitant administration of gentamicin with plant extract at the dose of 100 mg/kg significantly (p<0.01) prevented the decrease in SOD activity by 25.45 % in the kidney as compared to negative group. The treatment with gentamicin during 30 days induced significant decrease in catalase activity by 71.87 % (p<0.001) and 47.06 % (p<0.01) respectively in the liver and kidney as compared to NaCl group (Fig 1C). Concomitant administration of gentamicin with plant extract significantly prevented the decrease in catalase activity in the liver (p<0.01) and kidney (p<0.001) at the dose of 100 mg/kg. Treatment with gentamicin induced a significant decrease (p<0.001) in liver (52.14 %) and kidney (60.70 %) GSH concentration as compared to control group (Fig 1D). The extract administered with gentamicin prevented the decrease (p<0.001) in GSH concentration in liver and kidney at the dose of 100 mg/kg. At the dose of 200 mg/kg, the extract administered with gentamicin prevented the decrease in GSH concentration in liver (p<0.001) and in kidney (p<0.01) as compared to control group. Treatment with gentamicin induced a significant increase (p<0.01) in kidney nitrites concentration by 71.43 % as compared to control group (Fig 1D). The extract administered with gentamicin prevented the increase (p<0.001) in nitrites concentration in the kidney (55.10 %) at the dose of 100 mg/kg. Aspirin administered in the same condition significantly prevented the change in these parameters.

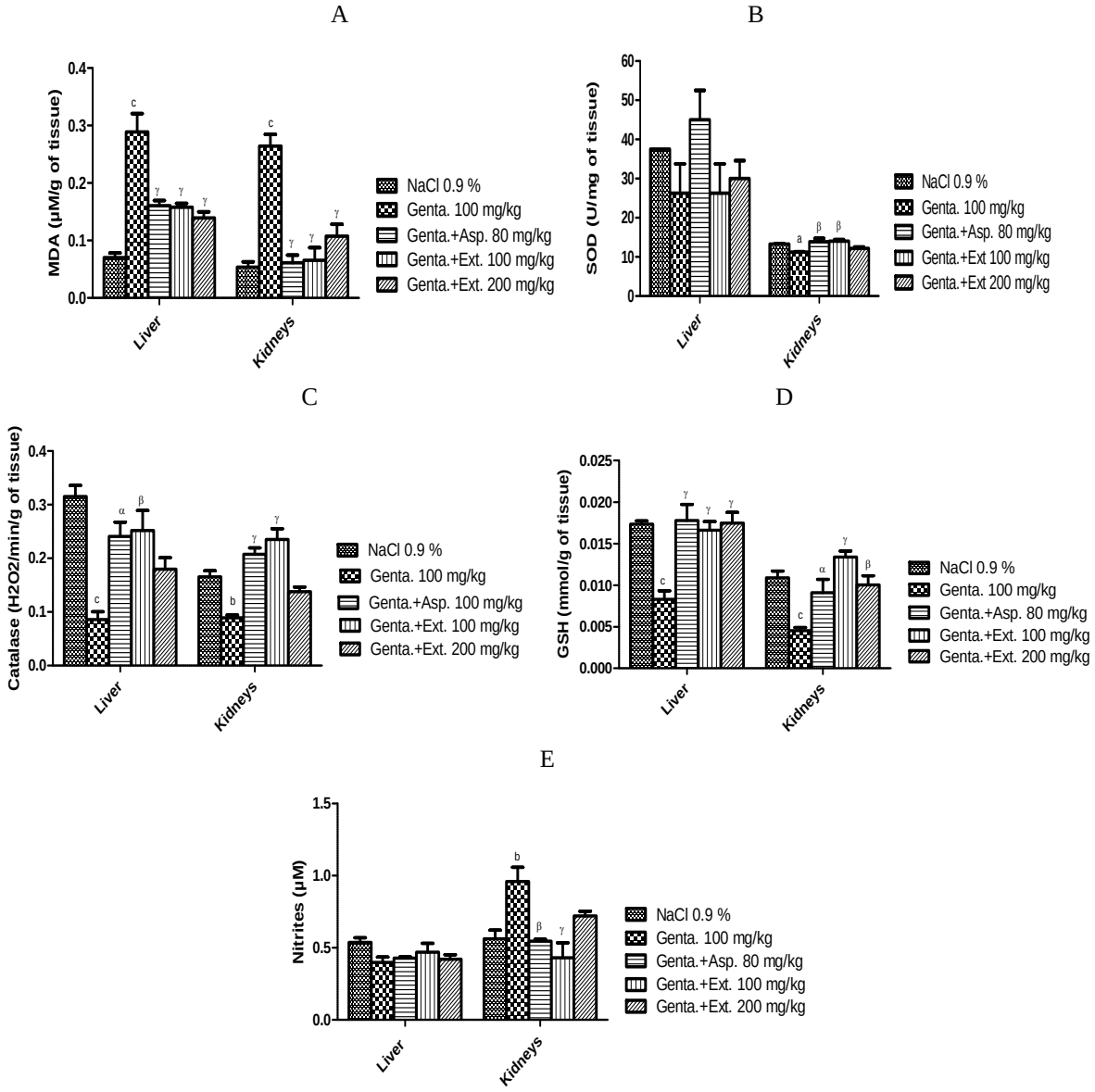

Figure 1: Effects of *E. indica* aqueous extract on some markers of oxidative stress

Each bar represents means ± S.E.M. of 5 rats ; [a]p<0.05, [b]p<0.01, [c]P<0.001 significantly different as compared to normal rats (NaCL 0.9 %). [α]p<0.05, [β]p<0.01, [γ]p<0.001 significantly different as compared to gentamicin group.

NaCl 0.9 %: sodium chloride 0.9 %, Genta. 100 mg/kg: gentamicin 100 mg/kg, Genta.+Asp. 80 mg/kg: gentamicin+aspirin 80 mg/kg, Genta.+Ext. 100 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (100 mg/kg), Genta.+Ext. 200 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (200 mg/kg), MDA: Malondylaldehyde ; SOD: superoxide dismutase ; GSH: reduced glutathione.

Effects of *E. indica* on arterial pressure and heart rate

Administration of gentamicin (100 mg/kg) for thirty days significantly increases systolic blood pressure (SBP) ($p<0.001$) by 45.51 %, diastolic blood pressure (DBP) ($p<0.05$) by 30.37 %, and mean blood pressure (MBP) ($p<0.001$) by 27.20 % as compared to normal rats (Figure 2). The administration of *E. indica* aqueous extract at the doses of 100 and 200 mg/kg significantly prevented the increase in SBP ($p<0.001$) by 28.72 % and 28.34 %, DBP ($p<0.05$) by 21.91 % and 24.94 %, and MBP ($p<0.01$) by 24.75 % and 26.35 % as compared to gentamicin-treated group. Aspirin (80 mg/kg) used in the same condition significantly prevented the change in SBP, DBP and MBP by 30.045 % ($p<0.001$), 27.52 % ($p<0.01$) and 28.57 % ($p<0.001$) respectively as compared to gentamicin group.

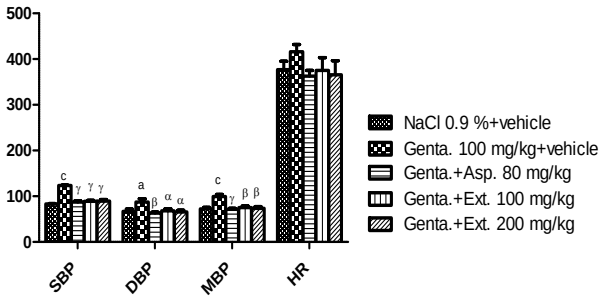

Figure 2: Effects of *E. indica* extract on arterial pressure and heart rate in different groups of rats.

Each bar represents means ±SEM of 5 rats. $^{a}p<0.05$, $^{c}p<0.001$ significantly different as compared to control rats. $^{\alpha}p<0.05$, $^{\beta}p<0.01$, $^{\gamma}p<0.001$ significantly different as compared to gentamicin group. NaCl 0.9 %: sodium chloride 0.9 %, Genta 100 mg/kg: gentamicin 100 mg/kg, Genta.+Asp. 80 mg/kg: gentamicin+aspirin 80 mg/kg, Genta.+Ext. 100 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (100 mg/kg), Genta.+Ext. 200 mg/kg: gentamicin+aqueous extract of *Eleusine indica* (200 mg/kg). SBP (systolic blood pressure), DBP (diastolic blood pressure) and MBP (mean blood pressure) in mmHg ; HR (heart rate) in beat/min.

Histological results

Figure 3 below shows photomicrographs of liver (A, B, C, D and E). Figure 3A shows normal hepatic tissue with normal architecture. Microscopic change of animal liver treated with gentamicin shows leucocytic cells infiltration, fibrosis and sinusoidal capillaries dilatation (Figure 3B). Liver of rats group received *E. indica* aqueous extract showed hepatic parenchyma close to those of normal group (Figures 3D and 3E).

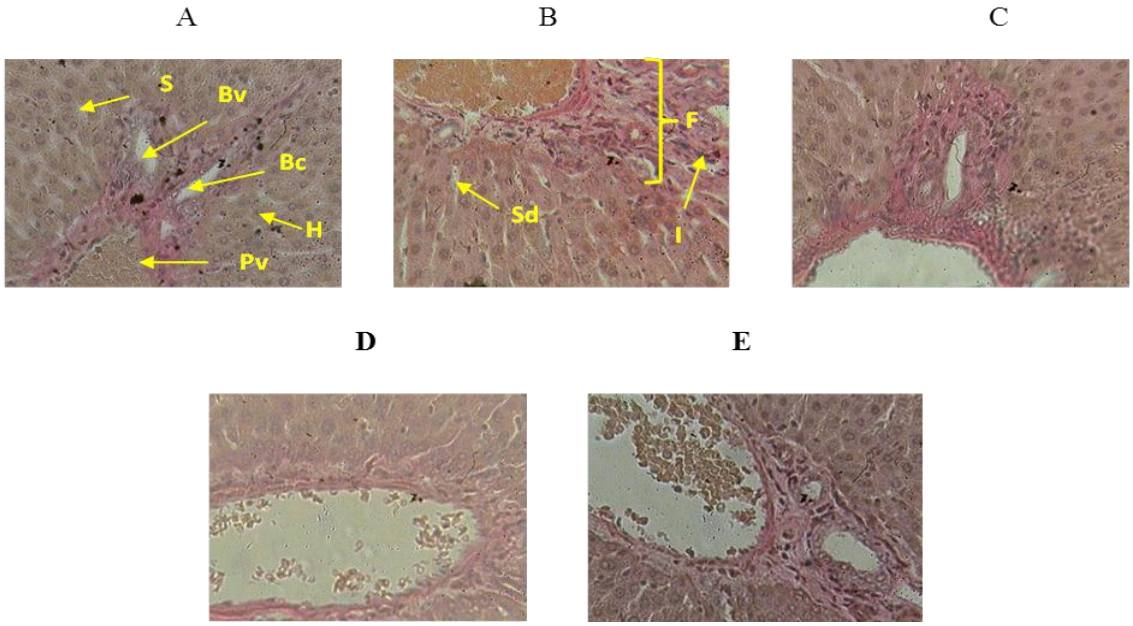

Figure 3: Photomicrograph of liver of NaCL 0.9 % group (A), gentamicin treated group (B), gentamicin and aspirin treated group (C), gentamicin and *E. indica* aqueous extract (100 and 200 mg/kg) (D and E). H: hepatocyte, S: sinusoïde, Pv: portal vein, Bv: biliary vein, Bc: biliary canal, I: leucocytic cells infiltration, F: fibrosis, Sd: sinusoidal dilatation (trichome of van Gieson×400).

Figure 4 below shows photomicrographs of kidney (F, G, H, I and J). Figure 3F shows normal renal tissue with normal architecture. Microscopic change of animal kidney treated with gentamicin shows fibrosis and leucocytic cells infiltration (Figure 4G). Kidney of rats group received *E. indica* aqueous extract showed renal parenchyma close to those of normal group (Figures 4I and 4J).

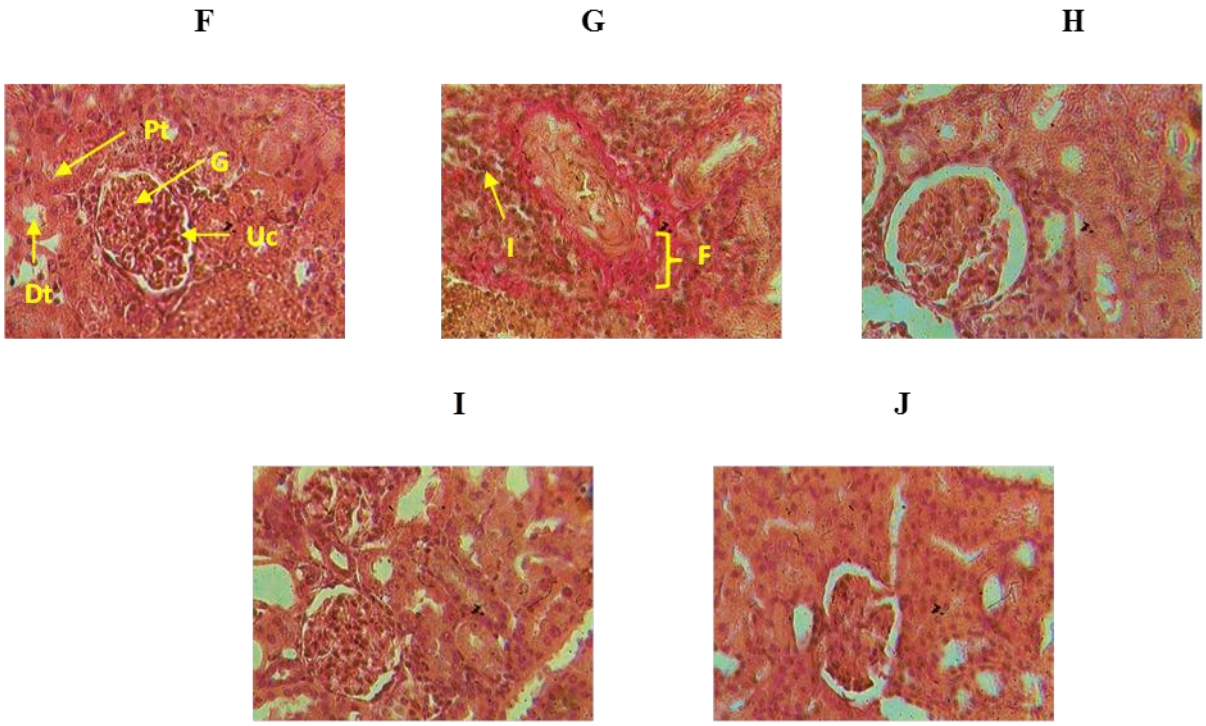

Figure 4: Photomicrograph of kidneys of NaCL 0.9 % group (F), gentamicin treated group (G), gentamicin and aspirin treated group (H), gentamicin and *E. indica* aqueous extract (100 and 200 mg/kg) (I and J), G: glomerulus, Pt: proximal tubule, Dt: distal tubule, Uc: urinary chamber, F: fibrosis, I: leucocytic cells infiltration (trichome of van Gieson×400).

Gentamicin is one of antibiotic used to prevent infection, however its have many harmful effects on some organs such as kidney and liver. Medical literature has described an increase incidence of acute hepatic and renal failure, especially with antibiotics use being the major cause among which the aminoglycoside such as gentamicin are most common. Hepato-nephrotoxicity represents a major health problem and accounts for incidence among population all over the world. The hepato-nephroprotective effects of *E. indica* aqueous extract against gentamicin-induced hepatic and renal toxicity in young rats were evaluated.

Gentamicin injected group in our study shown a significant increase in relative kidneys weight. These results are similar to those obtained by Ezejiofor *et al.*.[19] Any abnormal stimulus to the kidney or liver triggers an inflammatory response resulting in its increased weight.[20] The reduction or the increase in the internal organs weight is an indication of toxicity after exposure to a toxic substance.[21] Treatment with *Eleusine indica* aqueous extract was capable of reducing the hypertrophy of kidneys thus indicating that the treatment was capable of controlling the inflammation. Saponins and alkaloids contained in our extract are of great pharmaceutical importance because of their anti-inflammatory potential.[22]

In this study, there was significant increase in ALT and AST activities in the gentamicin treated group compared with NaCl 0.9 % group. Liver transaminases (ALT and AST) are important enzymes produced by the hepatocytes. The level of these enzymes in blood is increased in conditions in which the hepatocytes are damaged.[23] Rats treated with gentamicin shown a significant increase in serum level of total bilirubin. This increase could be explained by a hepatic attack which would result in the disturbance of the synthesis of biliary salts.[24] However, a reduction in ALT, AST and total bilirubin were observed in groups treated with *E. indica* aqueous extract (100 and 200 mg/kg). These reductions suggest the modulatory effects of the extract and its protective ability as a factor to decrease liver damage. *E indica* aqueous extract may contain bioactive substances which are able to protect the liver from the harmful effects induced by gentamicin. These effects could be due to the presence in this extract of the glycosides and phenols, compounds which act by stimulating the synthesis of the genes responsible of cellular regeneration.[25]

Gentamicin injection significantly affected lipid profile by enhancing the level of total cholesterol, triglycerides and LDL-cholesterol, and decreasing the level of HDL-cholesterol. In fact, the reduction of the activities of the lipoprotein lipase and triglyceride lipase enzymes, result in the decreased uptake of triglycerides from serum, causing its accumulation. The elevation of cholesterol level observed may be due to the increased activity of the enzyme β-hydroxymethylglutaryl CoA (HMGCoA) which catalyses the rate limiting step in cholesterol biosynthesis leading to increased cholesterol synthesis in tissues and excess leaking out of cholesterol into the blood.[26] The decreased of HDL may be due to the decrease of cholesterol ester tranfert protein (CETP) activity which transfers TG from VLDL to HDL.[26] HDL charged with TG are quikly hydrolysed and due to the fact of their higher catabolism, HDL blood level decreases and that of LDL increases.[27] *E. indica* aqueous extract administration improved the lipid profile, suggesting that this extract may allow restraining fat storage and dyslipidemia. Phytochemical studies revealed the presence of phenols and alkaloids, molecules whose hypolipidemic activities were shown. Indeed, phenols bind to cholesterol in the digestive tract in order to prevent their intestinal reabsorption and to increase their elimination.[28] Alkaloids stimulate hepatic catabolism of LDL to HDL and reduction in the level of LDL in favor of HDL leading to the reduction in cholesterol.[29]

Intraperitoneal injection with gentamicin for thirty consecutive days induced acute nephrotoxicity. Serum parameters such as creatinine, urea, uric acid, potassium ions were higher and sodium ions level was lower in gentamicin treated rats as compared to control NaCl 0.9 %. Our results are similar to those of Khattab.[30] Creatinine derives from endogenous sources, by tissue creatinine breakdown and its clearance enables a quite good estimation of the glomerular filtration rate.[31] A significant increase in creatinine level could possibly be a result of accumulation of gentamicin in the proximal tubular cells which are protonated in the body and bind to negatively charged phosphotidyl inositol. This binding causes the inhibition of lysosomal phospholipidosis. This excessive phospholipid overload causes proximal tubular necrosis.[32] Urea is the nitrogen containing end product of protein catabolism. The concentration of urea is elevated when glomerular filtration rate is markedly decreased in renal pathies. Moreover, urea concentration begins to rise only after parenchymal tissue damage. The possible reason behind the serum urea accumulation may be an increase rate of serum urea production than the clearance rate.[33] Uric acid is the end product of purine metabolism, hyperuricemia is associated with impaired renal function.[34] 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.[19] The co-administration of gentamicin and *Eleusine indica* aqueous extract exhibited a decrease in serum creatinine, urea and uric acid levels as compared to gentamicin treated group. An increase in serum potassium levels and a decrease sodium serum level were also observed gentamicin treated group as compared to NaCl group. The hyperkalemia is likely multifactorial and relates to inhibitory effects on Na-K-ATPase in collecting ducts and possibly to distal tubular acidosis.[35] The preventive role of *E. indica* aqueous extract was effective on Na^+ and K^+ serum level changes. It can be suggesting that this extract might interfere with mechanisms of gentamicin-induced injuries in kidney. Indeed, glycosides and phenols present in our extract can act by stimulating the synthesis of the genes responsible of cellular regeneration of renal tissue.[25]

Intraperitoneal injection of gentamicin during 30 days leads to the increase of systolic, diastolic and mean arterial blood pressure. Ibrahim *et al.*[36] reported that gentamicin administration causes elevation in blood pressure via the kidney damage, e.g. an interference of the renin-angiotensin system. Gentamicin stimulate the formation of reactive oxygen species such as H_2O_2 and $O_2°$ leeding to mesangial cells contraction witch stimulate the renin-angiotensin system, causing salt and water retention, thus generate hypertension.[37] The administration of aqueous extract of *E. indica* (200 mg/kg) led to the reduction in blood pressure in gentamicin treated rats. These results suggested that *E. indica* could act on some targets implicated in the genesis of hypertension such as vascular resistance, peripheral muscle tone, myocardiac contractility and volume overload to avoid hypertension. Indeed, our phytochemical results shown that the aqueous extract of *E. indica* contains saponins, alkaloids and phenols, which antihypertensive effects were shown, including endothelial integrity and relaxation of the muscular smooth vascular cells.[38]

MDA and nitrites levels were significantly increased, while the level of GSH, and the activities of catalase and SOD were significantly decreased in the kidney and liver tissues of gentamicin-treated rats as compared to control group, which indicated that gentamicin has caused severe oxidative stress. Gentamicin is known to increase the production of many ROS, such as superoxide and H_2O_2, which cause lipid peroxidation and subsequently oxidative tissue damage, by reducing the activities of renal antioxidant, SOD and catalase, in addition to depletion of GSH level [5]. In agreement with the present study, Christo *et al.*[39] reported that NO has a role in the acute renal failure caused by gentamicin because the free radical nature of NO might contribute to tubular damage. In addition, NO increases renal injury through its reaction with superoxide radical and generation of a cytotoxic peroxynitrite,[40] which could damage the tubular cells resulting in renal failure. Co-administration of gentamicin with *Eleusine indica* aqueous extract prevented the increase of MDA and nitrites, and the decrease of SOD, catalase and GSH levels induced by gentamicin, suggesting that this extract may prevent the

generation of free reactive oxygen species and the 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.[41]

Kidney sections from rats injected with GM showed severe changes as leucocytic cells infiltration and fibrosis. These results are similar to those of Khattab.[23] Liver sections from rats injected with gentamicin showed leucocytic cells infiltration, fibrosis and sinusoidal capillaries dilatation. Rats receiving *E. indica* aqueous extract showed appearance close to normal kidney and liver. This indicates that *E. indica* possess relative low adverse effects under the experimental conditions. The histopathological findings of the present study confirmed the obtained biochemical results, where oral administration of *E. indica* aqueous extract in gentamicin-treated rats considerably normalized the tested biochemical and bringing about remarkably recovery in kidney and liver as evidenced microscopically. These may be due to the antioxidant property of our extract, by increasing intake of antioxidants, avoided or minimized kidney injury by reducing oxidative stress.[34]

CONCLUSION

Gentamicin administration was characterized by dyslipidemia, increase in transaminases and total bilirubin. Gentamicin also induced kidney damages as evidenced by an increase in concentration of creatinine, urea, uric acid and K^+, with a decrease of Na^+ concentration. These abnormalities were associated with an increase in MDA and nitrites, and a depletion of GSH concentration, superoxide dismutase and catalase activities and histological damages. However concomitant administration of gentamicin with the plant extract improved hepatic parameters, lipid profile, renal parameters and antioxidant status. Thus, these results suggest that *Eleusine indica* aqueous extract exhibited hepato-nephroprotective effects. These effects might be related to its antioxidant potential and supports the traditional use of the whole plant of *Eleusine indica* to treat hepatic and renal problems.

REFERENCES

1. Pedraza-Chaverri J, Gonzalez-Orozco AE, Maldonado PD. Diallyl disulfide ameliorates gentamicin-induced oxidative stress and nephropathy in rats. Eur J Pharmacol, 2003; 473: 71-8.
2. Martinez-Salgado C, Henindez-Lopez FJ, Novoa-Lopez JM. Glomerular nephrotoxicity of aminoglycosides. Toxicol Appl Pharmacol, 2007; 223: 86-98.
3. Hall ME, do Carmo JM, da Silva AA, Juncos LA, Wang Z, Hall JE. Obesity, hypertension, and chronic kidney disease. Int J of Nephrology and Renovasc Disease, 2014; 7: 75-88.
4. Banday AA, Farooq N, Priyamvada S, Yusufi ANK, Khan F. Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane andoxidative stress in rat kidney tissues. Life Sciences, 2008; 82: 450-9.
5. Morsy MA, Ibrahim SA, Amin EF, Kamel MY, Rifaai RA, Hassan MK. Sildenafil ameliorates gentamicin-induced nephrotoxicity in rats: role of iNOS and eNOS. J of Toxicol, 2014; 2014: 1-8.
6. Heibashy MIA, Abdel MAE. Kidney and liver function tests after late dimethyl sulfoxide (DMSO) administration in rats with gentamicin induced acute renal failure. J Egypt Ger man Soc Zool, 1999; 30: 35-48.
7. Randjelovic P, Veljkovic S, Stojiljkovic N, Jankovic-Velickovic L, Sokolovic D, Stoiljkovic M, Ilic I. Salicylic Acid Attenuates Gentamicin-Induced Nephrotoxicity in Rats. The Sci World J, 2012; 6: 1-7.
8. Iqbal M, Gnanaraj C. *Eleusine indica* Linn. possesses antioxidant activity and precludes carbon tetrachloride (CCl4)-mediated oxidative hepatic damage in rats. Environ Health Prev Med, 2012; 17: 307-15.
9. Kulip JA. Preliminary survey of traditional medicinal plants in the west coast and interior of Sabah. J Trop For Sci, 1997; 10: 271-84.
10. De Melo GO, Muzitano MF, Legora-Machado A, Almeida TA, De Oliveira DB *et al*. C-glycosylflavones from the aerial parts of *Eleusine indica* inhibit LPS-induced mouse lung inflammation. Planta Med, 2005; 71: 362-73.
11. Tchoupou TH, Ngo LET, Ngueguim TF, Aboubakar BF, Njiaza J, Dimo T. Preventive effects of aqueous extract of the whole plant of *Eleusine indica* (Linn) Gaertn. (Poaceae) against L-NAME induced nephrotoxicity in rat. The J of Phytopharmacol, 2019; 8: 28-32.
12. Van Viet BN, Chage LL, Vladan LA, Schnyder-Candrian S, Montani JP. Direct and indirect methods used to study arterial blood pressure. J of Pharmacol and Toxicol Methods, 2000; 44: 361-73.
13. Friedewald WT, Levy RI, Fredickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. Clinical Chemistry, 1972; 18: 499-502.
14. Wilbur KM, Bernhein F, Shapiro OW. Determination of lipid peroxydation. Arch of Biochem and biophysics, 1949; 24: 3959-64.
15. Sinha AK. Colorimetric assay of catalase. Anal Biochem, 1972; 47: 389-94.
16. Ellman GL. Tissue sulfhydryl group. Arch Biomed Biophysics, 1959; 82: 70-7.
17. Misra HP, Fridovich I. Determination of the level of superoxide dismutase in whole blood. Yale Univ. Press New Haven, 1972; 101-9.

18. Green LC, Wagner DA, Glogowski J, Skippir PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate and nitrite in biological fluids. Anal Biochem, 1982; 126: 131-8.
19. Ezejiofor AN, Udowelle NA, Orisakwe OE. Nephroprotective and antioxidant effect of aqueous leaf extract of Costus Afer Ker gawl on cyclosporin-a (Csa) induced nephrotoxicity. Clin Phytosci, 2014; 2: 1-7.
20. Jayesh BD, Deepavali RT, Snehal NM, Archana RJ. Carissa carandas Linn. fruit extract ameliorates gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress. J of acute disease, 2015; 1: 135-40.
21. Witthawaskul P, Ampai P, Kanjanapothi D, Taesothikul N. Acute and subacute toxicities of the saponin mixture isolated fron Schejlera leucantha viguier. J Ethnopharmacol, 2003; 89: 115-21.
22. Sharma V, Verma RB, Sharma S. Preliminary evaluation of the hepatic protection by pharmacological properties of the aqueous extract of *Asparagus racemosus* in lead loaded swiss albino mice. Int J Pharm Pharm Sci, 2012; 4: 55-62.
23. Adedeji AL, Adedosu OT, Badmus JA, Adeleke GE, Afolayan IR, Olarinde IF. Aqueous extract of *Hibiscus Sabdariffa calyx* modulates gentamicin activity in rats. Asian Pac J Health Sci, 2016; 3: 178-87.
24. Zhen LM, Tao WY, Zou XL, Fu HZ, Ao ZH. Protective effects of mycelia of *Antrodia camphorate* and *Armillariella tabescens* in submerged culture against ethanol-induced hepatic toxicity in rats. J of Ethnopharmacol, 2007; 110: 160-4.
25. Rajendran R, Hemalatha S, Akasakalai K, M adkukrishra CH, Sohil B, Sundaram RM. Hepatoprotective activity of *Mimosa pudica* leaves against carbon tetrachloride induced toxicity. J of Nat Products, 2009; 2: 116-22.
26. Mozaffarian D. Fish, mercury, selenium and cardiovascular risk: Current evidence and unanswered questions. Int J Environ Res Public Health, 200; 6: 1894-916.
27. Trigatti B, Rigotti A, Kreiger M. The role of high-density lipoprotein receptor SRBI in cholesterol metabolism Curr. Opinion in Lipidol, 2000; 2: 123-31.
28. Fabrizio A, Delphine J. Role of liver in metabolism of lipoproteins. Hépato-Gastro, 2006; 13: 185-90.
29. Baliga MS, Jagentia GC, Ullo JN, Baliga MP, Venkatesh P, Reddy R, Baliga BS, Devi S, Raju SK, Veeresh V, Reddy TK, Biary B. Safety of Hydroalcoholic extract of sapthaparn (*Alstonia scholaris*) in mice and rats. Toxicology, 2004; 151: 317-26.
30. Khattab HAH. Effect of Morin against Gentamicin-Induced Nephrotoxicity in Young Male Rats. The Egyptian J of Hospital Med, 2012; 49: 705-17.
31. Shaheen U, Manzoor Z, Khaliq T, Kanwal A, Muhammad F, Javed HI, Munawar SH, Haq MI. Evaluation of Nephroprotective Effects of *Foeniculum vulgare* Mill, *Solanum Nigrum* Linn and their Mixture against Gentamicin-induced Nephrotoxicity in Albino Rabbits. Int J Pharm Sci Rev Res, 2014; 25: 1-9.
32. Lapkin R, Bowman R, Kaloyanides GE. J Pharmacol Exp Ther, 1999; 5: 201-33.
33. Safa J, Argani H, Bastani B, Nezami N, Ardebili BR, Ghorbanihaghjo A, Kalagheichi H, Amirfirouzi A, Mesgari M, Rad JS. Protective effect of grape seed extract on gentamicin induced acute kidney injury. Iranian J of Kidney Diseases, 2010; 4: 285-91.
34. Maarten N, Kuypers DRJ, Minnie S. Calcineurin inhibitor nephrotoxicity. Clin J of the Am Soc of Nephrol, 2009; 4: 481-508.
35. Ibrahim C, Stahlmann R, Merker HJ, Neubert D. Hypertension and nephrotoxic lesions in rats one year after prenatal exposure to gentamicin. Arch of Toxicol, 1988; 62: 274-84.
36. Ghafil FA, Al-Zubaidi FA, Almedeny SA. Assessment of Nephroprotective role of Irbesartan against gentamicin induced nephrotoxicity in rats. Kufa J For Veterinary Medical Science, 2012; 3: 54-60.
37. Lúcio RL, Viviane GP, Flávia MC, Aloa MS, Celso CN, Geovanni DC, Adelina MR, Maria GL, Maria AR. The effect of saponins from *Ampelozizyphus amazonicus* Ducke on the renal Na+ pumps' activities and urinary excretion of natriuretic peptides. BMC Complement and Altern Med, 2012; 12: 1-7.
38. Christo JS, Rodrigues AM, Mouro MG, Cenedeze MA, Simoes MJ, Schor N *et al*. Nitric oxide (NO) is associated with gentamicin (GENTA) nephrotoxicity and the renal function recovery after suspension of GENTA treatment in rats. Nitric Oxide, 2011; 24: 77-83.
39. Walker LM, Walker PD, Imam SZ, Ali SF, Mayeux PR. Evidence for peroxynitrite formation in renal ischemia-reperfusion injury: studies with the inducible nitric oxide synthase inhibitor L-N6-(1-iminoethyl)lysine). The J of Pharmacol and Experimental Therapeutics, 2000; 295: 417-22.
40. El-Sawi SA, Sleem AA. Flavonoids and hepatoprotective activity of leaves of Senna surattensis (burm.f.) in CCl4 induced hepatotoxicity in rats. Aust J Basic Appl Sci, 2010; 4: 1326-34.
41. Prahalathan P, Kumar S, Raja B. Effect of morin, a flavonoid against DOCA- salt hypertensive rats: a dose dependent study. Asian Pacific J of Trop Biomed, 2012; 2: 443-48.