# Hepatoprotective and antioxidant effects of Commelina diffusa Burm extracts on gentamicin-induced liver damage in rats

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#### **Abstract**

Commelina diffusa is a plant rich in antioxidant compounds, used in traditional medicine to treat many diseases. This study aimed to evaluate the hepatoprotective and antioxidant activities of aqueous and methanolic extracts of Commelina diffusa. Total phenolic and flavonoids content of extracts were determined. Hepatotoxicity was induced in rats by administration of gentamicin (80 mg/kg, i.p.) once daily for 10 days. Simultaneously, extracts were given at doses of 100 and 200 mg/kg, p.o. At the end of treatment, body weight of rats was measured; blood and liver tissues were collected for biochemical and histopathological analysis. The results showed that aqueous and methanolic extracts of C. diffusa contain significant amounts of total phenols and flavonoids. These extracts significantly decreased (p<0.05 to p<0.001) liver weight, activity of transaminases and alkaline phosphatase and the levels of malondialdehyde, bilirubin, LDL cholesterol, triglycerides and total cholesterol. In contrast they significantly increased (p<0.05 to p<0.01) the body weight of rats, the levels of HDL cholesterol, total protein, glutathione and the activity of catalase and superoxide dismutase. Histopathological examination revealed that extracts protected liver tissues against cell infiltration and centrolobular necrosis induced by gentamicin. These results suggest that Commelina diffusa could protect against hepatotoxic effects of gentamicin.

Keywords: Commelina diffusa, Gentamicin, Hepatoprotective activity, Antioxidant.

# Introduction

Liver is the largest organ of the body which performs several physiological functions. It plays an essential role in the metabolism of xenobiotics and many others foreign compounds that enter in the body. Because of this function, liver is often exposed to various diseases resulting in a number of clinical symptoms.<sup>2</sup> Liver disorders are the most common health hazard found in developed and developing countries due to dietary habits, chronic alcohol consumption, poor hygiene, smoking, toluene, carbon tetrachloride and use of drugs as diclofenac, paracetamol and gentamicin. Druginduced liver injury constitutes a major health problem. It represents more than 50% of the acute liver failure according to the United States Acute Liver Failure study group.<sup>3</sup> About 30% of patients treated with gentamicin are concerned with hepatotoxicity after a long period of treatment.<sup>4</sup> Liver diseases have become one of the major causes for morbidity and mortality in humans and animals all over the world. Herbal drugs have gained importance and popularity in recent years

because of their safety, efficacy and cost effectiveness.<sup>5</sup> Plant materials are used as hepatoprotective drugs because most of them are inexpensive, because of their better cultural acceptability, their better compatibility with the human body and their minimal side effects. These herbal drugs have shown the ability to maintain the normal functional statues of the liver with or without fewer side effects.<sup>6</sup> It has been reported that 160 phytoconstituents from several plants have hepatoprotective activities.<sup>7</sup>

Commelina diffusa Burm, sometimes known as the climbing day flower or spreading day flower is a pantropical herbaceous plant in the dayflower family Commelinaceae. This herb is widely distributed throughout America, South Asian countries and in Africa. In chinese traditional medicine, it is used to reduce swelling and inflammation. In different countries of Asia, Africa and America, it is commonly used in urinary tract infections, to remove cough with sticky phlegm and in diarrhoea, haemorrhoids, enteritis, eye irritation, conjunctivitis and other eye problems like

ophthalmia. <sup>9</sup> *C. diffusa* extracts contain a high proportion of concentrated antioxidants, which are derivatives of polyphenols. <sup>8</sup> Antioxidant properties, free radical scavenging abilities and anti-inflammatory activities are the basis of other bioactivities and health benefits, such as anti-aging, anticancer, and defensive activity for cardiovascular diseases, diabetes mellitus, obesity, neurodegenerative and hepatic diseases. <sup>10</sup> Therefore, the present study was conducted to evaluate the hepatoprotective and antioxidant effects of *C. Diffusa* extracts in rats intoxicated with gentamicin.

# Materials and Methods Drugs and chemicals

Different chemicals and solvents used in this study were of analytical grade. They were purchased from Geochim Laboratory Ltd, Bafoussam, Cameroon. Gentamicin and Silymarin were procured from the local pharmacy and the District Hospital of Dschang, Cameroon.

#### Plant material

The whole plant was collected at Dschang, West region of Cameroon in February 2017. The plant was authenticated at the National Herbarium of Cameroon by comparison to the voucher specimen registered to number SRFC/35189.

#### **Extraction procedure of plant materials**

The plant was dried at room temperature (24°C-25°C) away from sun light and ground into a fine powder using an electric blender. This powder was used to prepare aqueous and methanolic extracts.

#### Preparation of aqueous extract

Aqueous extract of *Commelina diffusa* (AECD) was obtained by decoction of 500g of powder in 5L of distilled water for 40 min. The decoction was then filtered with a whatmann  $N^{\circ}1$  paper (pore size  $\mu$ m). The filtrate was evaporated in a ventilated oven heated at 40°C to obtain 59 g paste. The yield extraction was 11.8%

# Preparation of methanolic extract

For this extraction, 400 g of powder of *C. diffusa* were macerated in 3L of methanol (95%) during 24h. After

this time, the macerate was filtrated and evaporated with a rotary evaporator under reduce pressure at 40-45°C temperature to obtain 13.6 g paste (the yield of extraction was 3.46%). Methanolic extract of *Commelina diffusa* (MECD) obtained was concentrated and stored in desiccators for further use.

The plant extracts (aqueous and methanolic) were dissolved in distilled water upon administration to obtain various concentrations that were administered to each animal using endoesophagus borer.

## **Determination of total phenolic content**

Total phenolic content of *C. diffusa* extracts was determined by the method described by Kodjio *et al.*<sup>11</sup> using Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard. The reaction mixture consisted of 20  $\mu$ L of extracts, 1380  $\mu$ L of distilled water, 200  $\mu$ L of 2N Folin-Ciocalteu Reagent and 400  $\mu$ L of 20% sodium carbonate solution. The mixture was incubated at 40°C for 20 min. After cooling, the absorbance was measured at 760 nm. The content of the sample was expressed as mg of GAE (gallic acid equivalent)/mg of extract.

#### **Determination of flavonoids content**

Flavonoid content of *C. Diffusa* extracts was determined by Aluminium chloride method described by Bendjaffer. The aqueous and methanolic extracts (100 μL) were mixed with 1.49 mL of distilled water and 30 μL of a 5% of NaNO<sub>2</sub> solution. After 5min, 30 μL of 10% of AlCl<sub>3</sub>H<sub>2</sub>O solution was added. After 6min, 200 μL of 0.1M sodium hydroxide and 240 μL of distilled were added. The solution was mixed and the absorbance was measured at 510 nm. The total flavonoid content of samples was expressed as mg of CE (Catechin equivalent)/mg of extract.

#### **Animals**

Forty-two (42) male's Wistar albino rats (12 weeks old) weighing between 200 to 220g were obtained from the Animal House of Department of Animal Biology of Faculty of Science, University of Dschang, Cameroon. They were maintained for a week in natural conditions of temperature and luminosity during which they were fed with standard food and had free access to drinking water. All these procedures were performed in

accordance with standard ethical guideline of the Laboratory of Animal Physiology and Phytopharmacology, Department of Animal Biology, University of Dschang, Cameroon.

# **Experimental design**

A total of 42 adults albino rats were divided into seven groups of six animals each. Group1 were considered as the normal control group and received distilled water. Group 2 or negative control group or hepatotoxic group received distilled water. Groups 3 and 4 were treated with AECD at doses of 100 and 200 mg/kg/day respectively. Groups 5 and 6 received MECD at doses of 100 and 200 mg/kg/day respectively. Group 7 were considered as positive control and received Silymarin (100)mg/kg/day). All these treatments administrated by oral route for 10 days. During these 10 days, all the animals received an intraperitoneal administration of gentamicin (GM) at dose of 80 mg/kg except the animals of the normal control group which received NaCl (0.9%).

## **Blood sample collection**

At the end of the experimental period, body weight of rats was measured. After this, rats were anesthetized by an intraperitoneal injection of Diazepam hydrochloride (5 mg/kg)/Ketamine hydrochloride (50 mg/kg). Blood was collected from each animal by catheterism of abdominal artery into non-heparinized test tubes and allowed to stand during 1 hour before centrifuging at 3000 rpm for 15 minutes. Serum was then collected and used for biochemical analysis.

After blood collection, liver was removed and weighed to determine relative liver weight. Then it was excised into two parts: one part was used for histopathological examination and the other part was crushed in 50 mM potassium phosphate buffer (pH 7.4) and the homogenate was used for antioxidant studies.

# **Biochemical Assay**

Estimation of biochemical parameters (alanine aminotransferase (ALT), aspartate aminotansferase (AST), alkaline phosphatise (ALP), total bilirubine

(TB), total proteins (TP), triglycerides (TG), total cholesterol (TC), HDL and LDL cholesterols was assayed using standard kids.

## **Antioxidant parameters**

Liver homogenates (5% w/v) were prepared in cold 50mM potassium phosphate buffer (pH 7.4). The unbroken cells and cell debris were removed by centrifugation at 1000 rpm for 10 min using a Remi refrigerated centrifuge. Subsequently, the homogenate was subjected to centrifugation (6000 rpm for 15 min) and the supernatant was used for the estimation of gluthation (GSH), malondialdehyde (MDA) and the activities of superoxide dismutase (SOD) and catalase (CAT).

## Histopathological studies

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol, cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin dye for photomicroscopic observation including cell necrosis, fatty change, hyaline regeneration and ballooning degeneration.

#### Statistical analysis

Results were expressed as the Mean  $\pm$  Standard Error Mean (S.E.M.) Data were analyzed by one way Analysis of Variance (ANOVA) using Graph Pad Prism versus 5.0. The means were separated and compared by post hoc-test of Turkey. p < 0.05 was considered as statistical significant.

#### Results

#### Total Phenols and flavonoids content

Both extracts contain a significant amount of phenolic and flavonoid compounds. However, the methanolic extract contains a higher amount of total phenolic content (18.53 mg of GAE/mg of extract) and flavonoids (1.99 mg of CE/mg) than the aqueous extract (Table 1).

Table 1: Total content of phenolic and flavonoid compounds

Extracts	Total phenolic content (mg of GAE / mg of extract)	Total flavonoid content (mg CE /mg of extract)
AECD	15.30	1.90
MECD	18.53	1.99

# Effects on body and liver weight of gentamicin-induced hepatotoxic rats

As presented in Table 2, gentamicin caused a significant decrease (p<0.001) in body weight and a significant increase (p<0.05) in liver weight of rats after 10 days of administration. Treatment with different doses of extracts and silymarin (100 mg/kg) significantly increased (p<0.05; p<0.01; p<0.001) the body weight of rats and significantly decrease (p<0.05; p<0.001) the liver weight compared to hepatotoxic group. Methanolic extract produced the best reduction (p<0.001) in liver weight.

**Table 2:** Effects of aqueous and methanolic extracts of *Commelina diffusa* on body and liver weight of gentamic induced hepatotoxic rats

Group/Treatment (mg/kg)	Relative body weight (%)	Relative liver weight (g/100gbw)
Normal control	123.00± 2.42	2.67±0.08
Negative control (GM group)	96.39±0.60°	3.09±0.14 <sup>a</sup>
AECD + GM	$106.10\pm0.79^{c\beta}$	2.42±0.04 <sup>γ</sup>
AECD 200 + GM	$104.70 \pm 1.36^{c\alpha}$	$2.50\pm0.05^{\gamma}$
MECD 100 + GM	$108.40 \pm 2.24^{c\gamma}$	2.32±0.09 <sup>γ</sup>
MECD 200 + GM	$106.20 \pm 1.62^{c\beta}$	2.32±0.12 <sup>γ</sup>
Silymarin 100 + GM	$112.40 \pm 1.40^{b\gamma}$	$2.54\pm0.05^{\beta}$

AECD= Aqueous extract of *Commelina diffusa*, MECD= Methanolic extract of *Commelina diffusa*. GM= Gentamicin, bw= body weight

Values are represented as Mean  $\pm$  S.E.M.

<sup>a</sup>p<0.05, <sup>c</sup>p<0.001, significantly different compared to the normal control group;

#### Effects on biochemical parameters of gentamicin-induced hepatotoxic rats

Effects of *C. diffusa* extracts in biochemical parameters on gentamicin-induced hepatotoxicity are resumed in Table 3. It appears that intraperitoneal injection of gentamicin significantly increased (p<0.01; p<0.001) the activities of ALT, AST, ALP, the levels of TB, TC and TG in rats. It did not cause any significant effect (p>0.05) on the level of TP and HDL and LDL cholesterols. *C. diffusa* extracts significantly decreased (p<0.05; 0.01; 0.001) the activities of ALT and ALP when compare to negative control group. Extracts also increased significantly (p<0.05 to 0.01) the level of TP and HDL-c compared to negative control group. In the other hand, *C. diffusa* extracts reduced significantly the levels of TB (p<0.05; p<0.01), TG (p<0.001) and LDL-c (0.01) when compare to negative control group.

**Table 3:** Effect of aqueous and methanolic extracts of *Commelina diffusa* on biochemical parameters of gentamicin-induced hepatotoxic rats

Parameters	Normal	Negative	AECD	AECD	MECD	MECD	Silymarin
	control	control	(100mg/kg)	(200mg/kg)	(100 mg/kg)	(200 mg/kg)	(100mg/kg)
ALT (IU/L)	29.67±3.90	57.00±4.38°	38.00±3.12α	33.33±3.36 <sup>B</sup>	38.33±4.31 <sup>α</sup>	27.00±3.08 <sup>γ</sup>	44.00±3.18
AST (IU/L)	67.73±9.53	138.16±6.5°	125.80±7.14°	113.16±7.4°	129.16±7.57 <sup>c</sup>	121.3±11.34°	160.0±0.17°
ALP (IU/L)	90.50±2.872	267.00±22.58°	109.20±7.68 <sup>γ</sup>	104.80±6.96 <sup>γ</sup>	199.80±18.66 <sup>γ</sup>	98.83±18.72 <sup>γ</sup>	$78.5 \pm 11.87^{\gamma}$
TP (g/dl)	7.72±1.1	2.10±0.18	21.92±6.66 <sup>aβ</sup>	6.14±0.79	16.97±2.60 <sup>α</sup>	12.03±2.66	10.50±3.60
TB (mg/dL)	0.90±0.13	1.40±0.15 <sup>b</sup>	1.17±0.05	$0.88\pm0.06^{\beta}$	1.00±0.06 <sup>α</sup>	1.21±0.03	1.36±0.06 <sup>b</sup>
TC (mg/dl)	10.50±0.34	14.00±1.98°	$3.83 \pm 0.40^{b\gamma}$	$3.50\pm0.22^{b\gamma}$	$4.16\pm0.70^{b\gamma}$	4.50±0.34 <sup>bγ</sup>	14.50±1.62
TG (mg/dl)	32.00±3.44	95.67±9.54 <sup>b</sup>	43.00±5.87 <sup>γ</sup>	16.33±4.41 <sup>γ</sup>	$21.16 \pm 3.13^{\gamma}$	42.33±8.67 <sup>γ</sup>	43.38±8.36 <sup>α</sup>

 $<sup>^{\</sup>alpha}$ p<0.05;  $^{\beta}$ p<0.01;  $^{\gamma}$ p<0.001, significantly different compared to the negative control.

HDL-C	1.99±0.04	1.74±0.08	2.26±0.09	$2.92\pm0.20^{\beta}$	2.36±0.22	2.81±0.21 <sup>β</sup>	2.82±0.11 <sup>β</sup>
(mg/dl)							
LDL-C	4.26±0.59	10.43±1.33	8.00±1.73	$2.00\pm0.36^{\beta}$	5.33±2.44	$3.66\pm1.05^{\alpha}$	$3.83\pm0.87^{\alpha}$
(mg/dl)							

AECD= Aqueous extract of Commelina diffusa, MECD=Methanolic extract of Commelina diffusa.

Values are represented as Mean  $\pm$  S.E.M.

<sup>b</sup>P<0.01; <sup>c</sup>P<0.001, significantly different compared to the normal control;

 $^{\alpha}P<0.05$ ;  $^{\beta}P<0.01$ ;  $^{\gamma}P<0.001$ , significantly different compared to the negative control.

# Effects on antioxidant biomarkers of gentamicin-induced hepatotoxic rats

As shown in Table 4, gentamicine significantly increased (p<0.05) the level of MDA and GSH, and significantly decrease (p<0.01) the activity of SOD and CAT compared to normal control group. However, treatment of rats with AECD (200mg/kg), MECD (100 and 200 mg/kg) and silymarin significantly decreased (p<0.05; p<0.01; p<0.001) the MDA and GSH levels. In addition, MECD and AECD at the dose of 200mg/kg significantly increased (p<0.05; p<0.01) the activities of CAT and SOD compared to hepatotoxic group. Only the dose of 100 mg/kg of MECD resulted in a significant increase (p<0.001) in CAT activity. In contrast, silymarin produced no significant effect on the activity of CAT and SOD.

**Table 4:** Effects of aqueous and methanolic extracts of *Commelina diffusa* on antioxidant parameters of gentamicin-induced hepatotoxic rats

Group/Treatment (mg/kg)	GSH	CAT	MDA (mol/mg	SOD	
	(nmol/g tissue)	(mol/mg tissue)	tissue)	(U/mg protein)	
Normal control	$0.145\pm0.002$	0.241±0.005	0.630±0.020	2.230±0.110	
Negative control	0.125±0.007a	0.152±0.004 <sup>b</sup>	0.960±0.070a	1.900±0.060 <sup>b</sup>	
AECD (100 mg/kg)	0.142±0.003	0.180±0.007	0.780±0.120	2.020±0.002	
AECD (200 mg/kg)	$0.148\pm0.000^{\beta}$	0.232±0.020 <sup>β</sup>	$0.550\pm0.060^{\beta}$	$2.160\pm0.020^{\alpha}$	
MECD (100 mg/kg)	$0.147\pm0.0003^{\alpha}$	$0.210\pm0.010^{\gamma}$	$0.680\pm0.040^{\alpha}$	2.040±0.020	
MECD (200 mg/kg)	$0.148\pm0.0003^{\alpha}$	0.201±0.020	$0.630\pm0.020^{\alpha}$	2.010±0.001	
Silymarin(100 mg/kg)	0.145±0.003 <sup>ß</sup>	0.202±0.020	$0,510\pm0.050^{\gamma}$	2.010±0.004	

AECD= Aqueous extract of Commelina diffusa, MECD=Methanolic extract of Commelina diffusa.GM= Gentamicin

Values are represented as Mean  $\pm$  S.E.M.

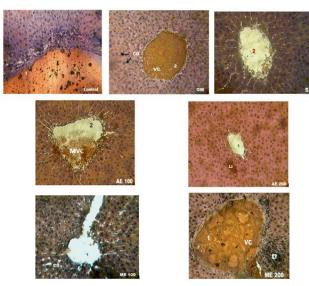
<sup>b</sup>P<0.01; <sup>c</sup>P<0.001, significantly different compared to the normal control group;

<sup>α</sup>P<0.05; <sup>β</sup>P<0.01; <sup>γ</sup>P<0.001, significantly different compared to the negative control.

# Effects on hepatic tissue of gentamicin-induced hepatotoxic rats

Histological sections of liver are presented in Fig. 1. This figure revealed the normal histology structures of liver in normal control group with normal hepatocytes, centrilobular vein and sinusoidal capillary (Fig. 1TM). In negative control group, significant changes were observed in liver structure with diffuse areas of vacuolar degeneration mainly centrolobular, swelling of cells and congestion of hepatic veins and sinusoids. Liver of rats treated with extracts at dose of 100 mg/kg

showed very little changes in hepatocytes; however, groups treated with AECD at dose of 200 mg/kg showed moderate vacuolar changes with hepatocytes swelling and lysis of cytoplasm. Liver's sections of rats treated with MECD (200 mg/kg bw), showed vascular congestion and leucocytes infiltration while the liver of those treated with 100 mg/kg of the same extract appeared normal.



**Figure 1:** Histological images of livers of rats pretreated with *Commelina diffusa* extracts and silymarin in gentamicin-induced hepatotoxicity (200x).

#### Control

Normal histological appearance cells with normal central and portal vein; GM or gentamicin group:capillary dilation and vascular congestion; SIL: liver section of rats treated with silymarin (100 mg/kg) showing well brought out central vein (CV) and hepatic cell with well-preserved cytoplasm; AE 100: liver section of rats treated with AECD (100 mg/kg) showing mild congestion in central vein; AE 200: liver section of rats treated with the AECD (200 mg/kg) showing regeneration of hepatocytes around central vein with near normal liver architecture; ME 100: liver section of rats treated with the MECD (100 mg/kg) showing capillaries dilation of sinusoids; ME 200: liver section of rats treated with the MECD (200 mg/kg) vascular congestion and leucocytes infiltration.

1: portal vein; 2: central vein; CD: capillary dilation; VD: vacuolar degeneration; I: infiltration of leucocytes; VC: vascular congestion; MVC: Mild vascular congestion

# **Discussion**

The present study was undertaken to evaluate the effects of aqueous and methanolic extracts of *C. Diffusa* on gentamicin induced hepatotoxicity in rats. Gentamicin is an antibiotic of the aminoglycoside class typically used for moderate to severe gram negative infections.<sup>13</sup> The aminoglycosides act by binding to the

aminoacyl site of 16S ribosomal RNA within the 30S bacterial ribosomal subunit, leading to misreading of genetic code which in turn alters protein synthesis of bacterial. Nowadays, the prolonged use of aminoglycosides, particularly gentamicin, becomes a fundamental part of medical practice. However its associated side effects seriously reduce it clinical use. It has been observed that most patients taking gentamicin encountered liver inefficiency problems. Gentamicin induces free radicals production, which can cause cell death via necrosis. The free radicals also activate the inflammatory response through nuclear factor kB (NFkB), inducing hepatotoxicity. In the serious production of t

In this study, GM treatment significantly reduced the body weight of rats. Similar results were obtained by Gaddam *et al.*<sup>17</sup> According to Shamsuyarova,<sup>18</sup> the recorded body weight loss could be attributed to dehydration and anorexia due to acidosis after administration of gentamicin. *C. diffusa* extracts improved growth performance; this effect could be attributed to the leaves content of the plant. Indeed, it is well known that, *C. Diffusa* leaves contain high crude protein, vitamins (A, B and C), phosphorus, iron, calcium.<sup>8,19</sup>

GM administration caused liver injury in rats as indicated by significant increase of ALT and AST activity and increase of liver weight. Increase of ALT and AST activity in gentamicin-induced liver damage is an indicator of hepatocellular leakage and loss of membrane integrity of liver cells.<sup>20,21</sup> It is well known that gentamicin causes liver damage through oxidative stress that occurs during its metabolism by hepatic microsomes, which in turn leads to lipid peroxidation of cell membranes.<sup>22</sup> Impairment of hepatic cell or membrane leads to discharge enzyme into circulation.<sup>23</sup> Hepatic damage observed in this study was confirmed by histological analysis which showed extensive vascular degenerative changes and centrolobular necrosis. Treatment with aqueous and methanolic extracts of C. diffusa and silymarin significantly decreased the ALT activity and liver weight meaning that they protected liver cells against gentamicininduced damage. Hepatoprotective effects of C. diffusa extracts were clearly established in histological analysis where rats treated particularly with AECD showed very

little changes of liver architecture when compared to normal control group.

GM significantly increased the serum liver markers function level such as bilirubin and ALP. Rise in serum activity of ALP is due to the increase in its synthesis, in the presence of an increase in biliary pressure due to the effect of a hepatotoxin.<sup>24</sup> Hyperbilirubinemia is generally associated with hemolysis and blockage of bile duct within the liver.<sup>25,26</sup> Both extracts significantly reduced these parameters.

Serum proteins level significantly decreased after chronic GM administration. Decrease of total protein levels is also considered as an index of the severity of hepatocellular dysfunction in chronic liver diseases. <sup>12</sup> In fact, when liver is sick, regulation of protein metabolism is frequently disturbed. One of clinical signs observed after disturbances in proteins metabolism is the low levels of plasma proteins synthesized in the liver. <sup>27</sup> Increase in TP observed in treated groups suggests that *C. diffusa* could restore the liver integrity and thus its metabolism function.

Chronic GM administration created serious disturbance of lipid profile characterised by a significant increase (p<0.01) of TC, TG, LDL-c and decrease of HDL-c in rats. According to Perla *et al.*<sup>28</sup> hyperlipidemia induced by gentamicin could reflect the impairment of liver cells to metabolize lipids or lipids peroxidation induction. Additionally, high levels of TG and LDL-C are associated with a high risk of cardiovascular diseases.<sup>29</sup> The use of *C. diffusa* extracts prevented the gentamicin induced rise in serum lipids and thus could prevent against cardiovascular diseases associated to liver dysfunction.

Most organisms have their antioxidant-based defence mechanisms against free radicals and reactive oxygen species (ROS) damage in which endogenous non-enzymatic and enzymatic antioxidants such as SOD, CAT, and GSH play an important role. SOD, GSH and CAT antioxidant constitute a mutually supportive team of defence against ROS.<sup>30</sup> The protective role of endogenous antioxidant system in humans is insufficient when free radical species become greater than antioxidant available.<sup>31</sup> In this case, free radical mainly superoxide radical, hydrogen peroxide, and hydroxyl radical induced oxidative stress with various consequences. In this study, GM significantly

reduced the GSH level, the activities of SOD and CAT with an increase of the MDA level. These results suggest that 10 days administration of gentamicin causes lipid peroxidation. According to Ozougwa and Eyo,<sup>31</sup> the decrease of the activities of antioxidant enzymes may lead to excessive cellular damage by free radical. Lipid peroxidation revealed in gentamicin group was significantly decreased in groups treated with extracts when compared to the negative control group. Simultaneously, significant increase of GSH, SOD and CAT was observed in groups treated with C. diffusa extracts and silymarin. SOD is the key defence enzyme that catalyses the conversion of superoxide radical to oxygen and peroxides. Peroxide is then metabolized by CAT into water and oxygen, protecting cells against free radical induced damage by reducing the amount of free radicals in the cells.<sup>32</sup> These results suggest that aqueous and methanolic extracts of C. diffusa possess antioxidant activity. This effect could be attributed to phenolic and flavonoid compounds contained in the plant. It is well known that these compounds are endowed with antioxidant properties.<sup>33</sup>-

Histopathological observations of liver sections showed capillary dilation and vascular congestion in GM group. These abnormalities were totally corrected in groups treated with silymarin and aqueous extract of *C. diffusa* at dose of 200 mg/kg, evidence of their protective effect against GM induced liver injuries.

#### Conclusion

The present study demonstrated the protective activity of *C. Diffusa* extracts against gentamicin induced hepatotoxicity. The results indicated that *C. diffusa* prevented gentamicin-induced liver damage by restoring altered biochemical and histological parameters and its antioxidant defences. These findings provide biochemical and histological data supporting folkloric use of *Commelina diffusa*Burm .f. in the local treatment of some hepatic disorders.

#### **Conflict of interest**

None.

#### **Conflict of Interest**

None.

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