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NEPHRO-PROTECTIVE AND HEPATO-PROTECTIVE PROPERTY OF COMMELINA DIFFUSA LEAF EXTRACT IN DOXORUBICIN-INDUCED ALBINO RATS.

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ABSTRACT

Ethanol leaf extract of commelina diffusa at 200 and 400mg/kg was administered to albino rats for 20 days and simultaneously treated with doxorubicin (cumulative dose of 15 mg/kg i.p. in six divided doses 2.5mg/kg body weight i.p of doxorubicin on 7th, 10th,13th, 16th and 19th day of study). On the 20th day, AST (Alanine amino transferase), ALT (Aspartate amino transferase), TB (total bilirubin), Urea and Creatinine were evaluated, using the various standard kits. Pretreatment with leaf extract of *Commelina diffusa* at 200 and 400 mg/kg showed a significant decrease in the levels of AST, ALT, TB, Urea and Creatinine when compared with the positive control (doxorubicin treated group) $p< 0.05$. The kidney sections obtained from doxorubicin treated animals showed, necrotic tubules with marked perforation and intense distortion of tissue architecture. Animals pretreated with extract of *Commelina diffusa* at 200 and 400 mg/kg showed normal kidney stroma with Bowman's capsule with glomerulus and renal tubules. Also, the liver sections obtained from doxorubicin treated animals showed infiltration of neutrophils with spotty inflammation. Animals pretreated with extract of *Commelina diffusa* at 200 and 400 mg/kg showed improvement in the cell integrity evidenced by normal hepatic stroma with hepatocytes, sinusoid and central vein. The results obtained from this study show that the ethanol leaf extract of Commelina diffusa may possess nephron-protective and hepato-protective functions against doxorubicin-induced wistar albino rats.

KEYWORDS: *Commelina diffusa*, Doxorubicin, Alanine amino transferase, Aspartate amino transferase, nephron-protective, hepatoprotective.

INTRODUCTION

Commelina L. is the largest genus of Commelinaceae, ranging between 170-215 species that are well distributed in the warmer regions of the world with tropical Asia having the greatest diversity.[1, 2, 3] *Commelina* is easily differentiated from the remaining genera in the tribe by its inflorescences which are subtended by spathaceous basal bracts and reduced to fasciculate zygomorphic flowers.[4] Commelina diffusa is a pantropical plant in the day flowering plant that is most commonly known as climbing day flower or spreading day flower. Commelina diffusa is found throughout the tropics of America, Africa, Asia and the Pacific, also in the subtropics in the southern part of the USA, South America, Australia and south Asian islands.[5] The weed is always an annual in temperate countries while being an annual or perennial in tropical and subtropical countries, depending on the moisture availability and is commonly found in damp shady places near water, open swamps and marshes.[6] They usually spread diffusely, creeping along the ground, branching heavily and rooting at the nodes, with a stem length of 1m.[7] The leaf blades are relatively variable, ranging from lanceolate to ovate, with proximal leaves tending to be more oblong. Flower colour is most typically blue, but lilac, lavender, yellow, peach, apricot and white.[8] Phytochemical screening of *Commelina diffusa* has reportedly indicated the presence of tannins, Saponins, alkaloids, flavonoids and glycosides with the absence of anthraquinones, sterols and phenols.[9] Commelina diffusa has been used for various purposes ranging from food, industrial purposes and medicinal benefits. The young leaf shoot can be eaten directly as vegetable or use fresh in salads. Commelina diffusa can be prepared for medicinal purpose by the plant been infused, decocted, juiced or steamed. It can be effective used for the management of cardiovascular, digestive, head and throat, hepatic, muscloskeletal and tumor related diseases. The flower of commelina diffusa can be used for paint and cosmetics purpose.[8,10] Due to the enormous importance of the plant as much in feeding as in therapeutic uses, the present study was carried out to ascertain the nephro-protective and hepato-protective activity of ethanol leaf extract of *Commelina Diffusa* on doxorubicin-induced albino rats.

MATERIALS AND METHOD

2.1 Animals

Twenty four (24) male wistar albino rats weighing between 110g to 130g were obtained from the Department of Pharmacology Animal House of Niger Delta University Bayelsa State and were acclimatized for one week during which they were fed with standard feed (pellet) and distilled water. All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) as per the direction of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

2.2 Chemicals/Reagents

Chloroform, absolute ethanol (was product of BDH Chemical Company Ltd, Poole, England), distilled water, normal saline, formaldehyde, Doxorubicin (Khandelwal Laboratories Pvt Ltd, Mumbai, India). AST, ALT, Urea, Creatinine and Bilirubin Kits were from Randox Laboratories Ltd, Co. Antrim, United Kingdom. Sigma-Aldrich Ltd., U.S.A. PerkinElmer, USA, Rat chow was purchased from Pfizer Nigeria Plc. All other reagents/chemicals obtained from standard suppliers were of analytical grade.

2.3 Collection of extract /extraction procedure

Fresh leaves of *Commelina diffusa* were collected from Amassoma town, Bayelsa State, Nigeria. The plant was identified by a botanist Prof. K. Ajibeshin, Department of Pharmacognosy, Faculty of Phamarcy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria and deposited at the Herbarium of Department. The leaves were plucked off their stems and air dried at room temperature for one week. The dried leaves were ground to powder using a blender. Three hundred and ten grams (310g) of the powdered materials was mixed with 500ml of absolute ethanol and 500ml of distilled water, at a ratio of 1:1. Mixture was macerated for three days (72 hours) with constant agitation (shaking) to ensure proper mixing and extraction. The mixture was filtered into 1000ml beaker using glass filter and filter paper and the filtrate was evaporated to dryness in a water bath for three days at 40^{o}C to obtain 47.69g paste. Appropriate weights of the residue were prepared with normal saline water to obtain various concentrations that were administered to each of the rats.

2.4 Experimental design and procedures

A total of 24 adult albino rat strains grouped into four each having six rats grouped as follows;

Group 1: (Normal Control): (200ml/kg body weight saline water and pellet feed for 20 days.

Group 2:(positive control): received 2.5mg/kg body weight i.p of doxorubicin on 7th, 10th,13th, 16th, and 19th days.

Group 3:(test group): received 200mg/kg/bodyweight of *C. diffusa*, i.p for 20 days+ 2.5mg/kg body weight i.p of doxorubicin on 7th, 10th,13th, 16th, and 19th days.

Group 4: (test group): (test group): received 400mg/kg/bodyweight of *C. diffusa*, i.p for 20 days+ 2.5mg/kg body weight i.p of doxorubicin on 7th, 10th,13th, 16th and 19th days.

Animals were sacrificed on the 20th day of study. Blood was collected into plain bottles for various biochemical analyses. The liver and kidney were harvested and fixed in 10% formalin for histological study.

2.5 Sample Collection and Biochemical Analysis

At the end of the experimental period, the rats were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. Blood was collected from each animal by cardiac puncture using sterile needle and syringe into well labeled plain tubes, allowed to stand for 2hrs before centrifuging at 3000rpm for 10 minutes. The supernatant was used for the biochemical analysis. The liver and kidney were excised using a midline abdominal incision, weighed and transferred into 10% neutral buffered formalin for histopathological examination.

2.6 Biochemical Assay

Serum transaminase (ALT and AST) was determined by method of.[11] Bilirubin was estimated by colorimetric method.[12] Serum Urea was estimated by[13] method and serum creatinine by.[14]

2.7 Statistical Analysis

All data were expressed as Mean ± Standard deviation. The data obtained were analyzed using Two-way Analysis of Variance (ANOVA) using SPSS (Statistical Package for Social Sciences) Version 20. The means were separated and compared by post-Hoc and Turkey method. $P < 0.05$ was considered as statistical significant.

3.0 RESULTS

Effect of ethanol extract of *Commelina diffusa* leaf on doxorubicin-Induced Albino Rats Body weight, liver and kidney weight, liver and kidney/body weight ratio.

Decrease in mean body weight, mean liver weight, mean kidney weight, liver/body weight ratio and kidney body weight ratio was seen in doxorubicin treated rat, at the end of the study

when compared with the normal control. Extract of Commelina diffusa at 200 and 400 mg/kg, show significant increase in mean liver and kidney weight, increase in kidney/body weight ratio when compared with doxorubicin control group, ($p< 0.05$). (Table 1).

Biochemical study

Serum Liver Markers: AST, ALT, Bilirubin

Treatment with doxorubicin causes an elevation in level of ALT, AST and Bilirubin, which are considered as the selective biomarkers of hepatic damage when compared with the normal control. Our study showed decrease in the elevated levels of these enzymes. Pretreatment with extract of Commelina diffusa at 200 and 400 mg/kg showed a dose dependent, significant decrease in ALT, AST and Bilirubin ($P<0.05$) when compared with the doxorubicin control group. (Table 2).

Serum Kidney Markers: Urea, Creatinine

Doxorubicin treated group, showed increase levels of Urea and Creatinine, which are indicative biomarkers of nephrotic damage when compared with the normal control. The present study, indicated that pretreated groups with extract of Commelina diffusa at 200 and 400mg/kg significantly decrease the level of urea and creatinine in a dose dependent manner, ($P<0.05$), when compared with the positive control group. (Table 2).

Table 1: Effect of ethanol extract of Commelina diffusa leaf on body weight, Liver weight and Kidney weight on doxorubicin-induced Albino Rats.

Group/Treatment	Mean wt (g) Rats day 1	Mean wt (g) Rats day 21	Mean wt (g) Rat Liver	Mean wt (g) Rat Kidney	Mean wt Liver/body ratio X 10^{-3}	Mean wt Kidney/body ratio X 10^{-3}
Control group Saline water	121 ± 8.9^{a}	193 ± 12.1^{b}	4.67 ± 2.1^{d}	1.05 ± 0.5^{f}	24.2 ± 0.17^{i}	5.4 ± 0.40^{k}
Positive control 2.5mg/kg DOX	120 ± 8.9^{a}	160 ± 9.9^{c}	4.49 ± 3.9^{e}	0.41 ± 0.1^{g}	28.1 ± 0.39^{j}	2.6 ± 0.01^{L}
Test group 200mg/kg extract & DOX	119 ± 8.4^{a}	158 ± 9.4^{c}	4.58 ± 3.4^{d}	0.71 ± 0.2^{h}	28.9 ± 0.36^{j}	4.5 ± 0.02^{k}
Test group 400mg/kg extract &DOX	113 ± 8.0^{a}	168 ± 10.4^{c}	4.79 ± 4.0^{d}	0.77 ± 0.3^{h}	28.5 ± 0.38^{j}	4.6 ± 0.03^{k}

Values are represented as Mean ± SD. Value with different superscripts from control are statistically different at $p<0.05$.

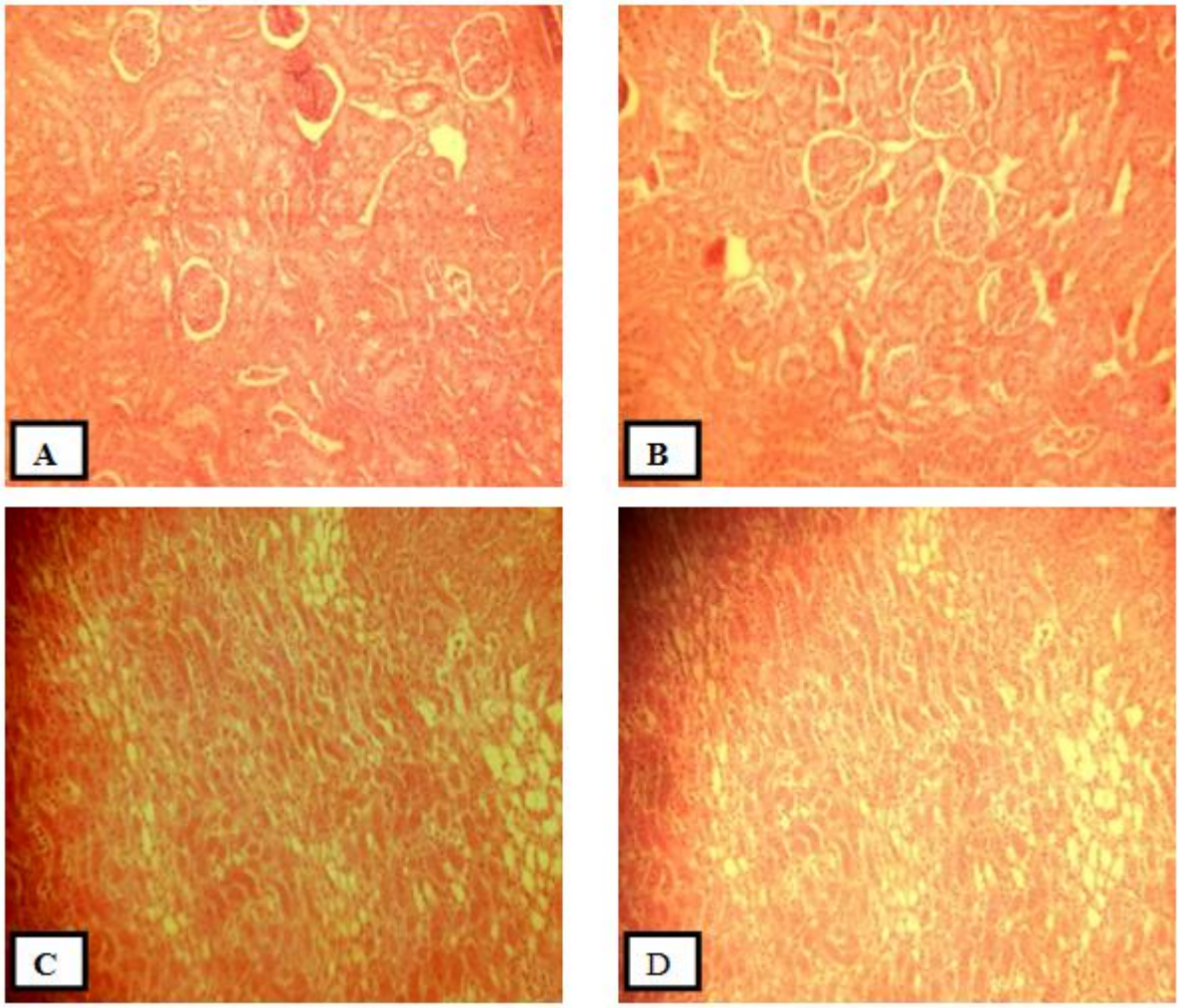
Table 2: Effect of ethanol extract of Commelina diffusa leaf on AST, ALT, Bilirubin, Creatinine and Urea levels in doxorubicin-induced Albino Rats.

Groups	UREA (Mmol/l	BILIRUBIN (µmol/l)	CREATININE (Mg/dl)	AST (U/L)	ALT (U/L)
Control					
Group	70.90±7.3[a]	25.09±3.2[a]	9.28±1.3[a]	83.50± 0.03[a]	20.21±4.2[a]
saline					
water					
Positive					
control	79.60±6.8[b]	50.08±4.6 [b]	36.70±3.9[b]	147.45±10.2[b]	76.4±8.5[b]
2.5mg/kg					
DOX					
Test					
Group	66.60±4.7[a]	38.30±2.8[a]	12.20±2.3[a]	116.72±7.2[c]	54.4±7.9[c]
200mg/kg					
Extract &					
DOX					
Test					
group	58.10±4.1[c]	9.54±1.4[c]	29.60±3.1[c]	89.52±5.9[a]	28.1±6.3[a]
400mg/kg					
Extract &					
DOX					

Values are represented as Mean ± SD. Value with different superscripts from control are statistically different at $p<0.05$.

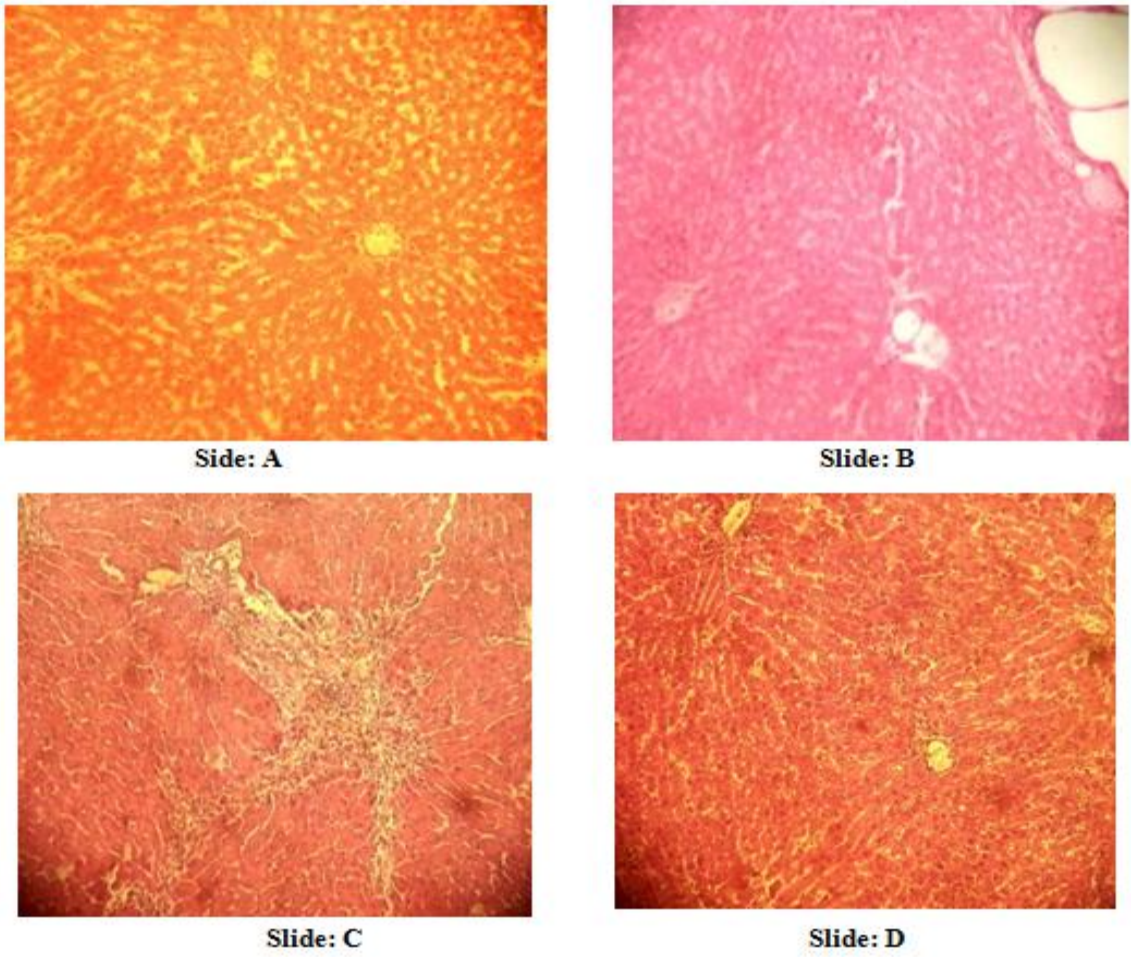
Histopathological changes on Doxorubicin-induced Albino Rats

The kidney sections obtained from doxorubicin treated animals showed, necrotic tubules with marked perforation and intense distortion of tissue architecture. Animals pretreated with extract of *Commelina diffusa* at 200 and 400 mg/kg showed normal kidney stroma with Bowman's capsule with glomerulus and renal tubules. (Fig. 1). The liver sections obtained from doxorubicin treated animals showed infiltration of neutrophils with spotty inflammation. Animals pretreated with extract of *Commelina diffusa* at 200 and 400 mg/kg showed improvement in the cell integrity evidenced by normal hepatic stroma with hepatocytes, sinusoid and central vein (Fig. 2).



Haematoxylin and Eosin, X 100

Fig. 1: Histopathological images of kidney pretreated with ethanol extract of Commelina diffusa in doxorubicin-induced albino rats. A: Normal control, B: Positive control, C: Test group – 200 mg/kg, D:Test group – 400 mg/kg.



Haematoxylin and Eosin, X 100

Fig. 2: Histopathological images of liver pretreated with ethanol extract of Commelina diffusa in doxorubicin-induced albino rats. A: Normal control, B: Positive control, C: Test group – 200 mg/kg, D: Test group – 400 mg/kg.

DISCUSSION

The use of a variety of natural and synthetic antioxidants to prevent doxorubicin's toxicity has been considered. The combinations of doxorubicin with agents capable of blocking its reactive oxygen species mediated toxicity effect has been investigated.[15] The present study was carried out to investigate the biochemical effect of ethanol extract of Commelina diffusa leaf in doxorubicin-induced albino rats by using liver and kidney serum marker enzymes. Oxidative stress is the consequence of a disturbed prooxidant/antioxidant intracellular balance, due to abnormal production of reactive oxygen species (ROS) or depletion of antioxidant defenses. Continuative oxidative stress leads to cell damage, which results in the alteration of cellular enzymes.[16] Oxidative stress and mitochondrial dysfunction are associated with disease and toxic process. It results from over production of reactive oxygen

species, often leading to peroxidation of membrane phospholipids and production of reactive aldehydes.[17] In this study, treatment with doxorubicin causes a significant increase in the level of AST, ALT and total bilirubin when compared with the normal control. Our study demonstrated a significant decrease in the level of AST, ALT and total bilirubin in the *Commelina diffusa* extract treated groups when compared with the positive control. These results indicate the hepatoprotective effect or free radical scavenging effect of the extract of *Commelina diffusa* in oxidative damage caused by doxorubicin. Lipooxygenase inhibition of methanol leaf extract of Commelina benghalensis was reported and the extract showed significant lipooxygenase inhibition due to the presence of flavonoids, which have been responsible for its inhibitory activity.[18] The presence of antioxidant constituents such as flavonoids and tannins might be responsible for the free radical scavenging and antioxidant activity of the extract. Presence of tannins, saponins, alkaloids, flavonoids and glycosides has been reported during preliminary study on phytochemical screening of the plant.[9] The leaf extract of *Commelina diffusa* has been shown also, to possess strong antioxidant and anti-inflammatory properties.[19] Alcohol root extract of Commelina benghalensis was earlier reported to show significant hepatoprotective activity in paracetamol-induced liver damage model in wistar rats.[20]

Doxorubicin treated group, showed increase levels of Urea and Creatinine, which are indicative biomarkers of nephrotic damage when compared with the normal control. The present study, indicated that pretreated groups with extract of Commelina diffusa at 200 and 400mg/kg significantly decrease the level of urea and creatinine in a dose dependent manner, ($P<0.05$), when compared with the positive control group. Renal diseases which diminish the glomerular filtration lead to urea retention and decrease in urea excretion is seen in severe liver disease with destruction of cells leadings to impairment of the urea cycle.[21] Creatinine is a waste product formed in muscle by creatinine metabolism. Creatinine is synthesized in the liver, passes into the circulation and is taken up by skeletal muscle. Its retention in the blood is evidence of kidney impairment. Our results is in line with the report by[22], that leaf extracts of Commelina benghalensis and Cissus quadrangularis showed nephron-protection from nephrotic damage caused by quinalphos in rats by restoring the level of kidney markers. The presence of antioxidant constituents such as flavonoids and tannins might be responsible for the nephroprotectivity of the extract.

Decrease in mean body weight, mean liver weight, mean kidney weight, liver/body weight ratio and kidney body weight ratio was seen in doxorubicin treated rat, at the end of the study when compared with the normal control. Extract of Commelina diffusa at 200 and 400 mg/kg, show significant increase in mean liver weight, mean kidney weight and increase in kidney/body weight ratio when compared with doxorubicin control group. The results in this study is in accordance with other studies[23] which may be attributed to reduced food intake and inhibition of protein synthesis due to doxorubicin treatment compared to normal control.

The kidney sections obtained from doxorubicin treated animals showed, necrotic tubules with marked perforation and intense distortion of tissue architecture. Animals pretreated with extract of *Commelina diffusa* at 200 and 400 mg/kg showed normal kidney stroma with Bowman's capsule with glomerulus and renal tubules. Also, the liver sections obtained from doxorubicin treated animals showed infiltration of neutrophils with spotty inflammation. Animals pretreated with extract of *Commelina diffusa* at 200 and 400 mg/kg showed improvement in the cell integrity evidenced by normal hepatic stroma with hepatocytes, sinusoid and central vein. The results obtained from this study show that the ethanol leaf extract of Commelina diffusa may possess nephron and hepato-protective functions against doxorubicin-induced wistar albino rats.

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