

Research Article

Hepatoprotective and nephroprotective activities of *Solenostemon monostachyus* P. Beauv (Lamiaceae) leaf extract

Jude E. Okokon ^a, Wilfred N. Oguamanam ^a, and Emem E. Umoh ^a

^a *Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria*

* **Corresponding author:** Department Of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, P.M.B 1017, Uyo, Nigeria. Tel: +234-802-3453678. Email: judefiom@yahoo.com

Background: *Solenostemon monostachyus* P. Beauv (family Lamiaceae), a medicinal herb in West and Central Africa, is ethnomedically employed as an antidote for poison and for the treatment of different diseases and ailments.

Objective: To investigate the hepatoprotective and renoprotective effects of *S. monostachyus* leaf extract against paracetamol- induced liver and kidney injuries in rats.

Methodology: Hepato-renoprotective effects of *S. monostachyus* ethanol leaf extract was evaluated against paracetamol- induced liver and kidney injuries in rats. The liver protective property of the ethanol leaf extract (75-225 mg/kg) was investigated by the assessment of liver function parameters, liver antioxidant enzymes and histopathology, while the renoprotective property was evaluated by the assessment of some kidney function parameters, kidney antioxidant enzymes and histopathology. Silymarin (100mg/kg) was used as positive control.

Results : The leaf extract exerted significant ($p < 0.05 - 0.001$) dose-dependent decreases in elevated levels of liver enzymes (ALT, AST and ALP), total cholesterol, direct and total bilirubin as well as increases in serum levels of total protein, albumin and antioxidant enzymes (SOD, CAT, GPx) and GSH. Histopathological study of the liver sections of extract and silymarin-treated rats revealed reductions in the pathological features compared to the paracetamol-treated animals. Leaf extract pre-treatment also resulted in significant ($p < 0.05$) dose-dependent decreases in increased levels of serum creatinine and urea without affecting the electrolytes levels. Histopathology of the kidney sections of extract and silymarin- treated rats showed decreases in the pathological features compared to the control group. The chemical pathological results in both liver and kidney agreed with histopathological observations indicating pronounced hepatoprotective and renoprotective effect of the leaf extract of *S. monostachyus*.

Conclusion: The results of this study demonstrate that the leaf extract of *S. monostachyus* has the potentials to protect the liver and kidney against injury which may be due to its antioxidant activity of its constituents and this can be employed in the management of liver and kidney diseases.

Keywords: *Solenostemon monostachyus*, medicinal plant, hepatoprotective, renoprotective, antioxidant.

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1. Introduction

Solenostemon monostachyus P. Beauv (family Lamiaceae) is an herb widely distributed in West and Central Africa. It is a succulent annual weed predominant in anthropogenic habitats and rocky

savannahs and grows up to 100 cm tall (Mve-Mba et al, 1994). The aerial parts are employed ethnomedically by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, fever/malaria (Ajibesin et al, 2008; Adebayo and Krettli, 2011), and other diseases. The plant is used as a diuretic as well as to treat

hypertension (Koffi et al, 2009). The plant has been found to contain essential oil (Mve-Mba et al, 1994), diterpenoids (Toshio et al, 1980), flavonoids, coumarin and polyphenol (Datte et al, 2010; N'guessan et al, 2011). The essential oil contains; β -pinene, oct-1-en-3-ol, β -caryophyllene, octan-3-ol and (*E,E*)- α -farnesene (Mvé-Mba et al, 1994). Previous works on the plant include; antioxidant (Datte et al, 2010; N'guessan et al, 2011; Okoko and Ere, 2012), antihypertensive (Fidele et al, 2012) and antimicrobial activities (Ekundayo and Ezeogu, 2006), antipyretic and antiplasmodial (Okokon et al, 2016a), analgesic and anti-inflammatory (Okokon et al, 2016b). This investigation was carried out to evaluate the hepatoprotective and nephroprotective effect of *Solenostemon monostachyus* and support its use in traditional medicine to treat liver and kidney disorders.

2. Materials and Methods

2.1 Plant collection

The plant material, (*S. monostachyus* aerial parts) were collected in a farm area in Uyo, Akwa Ibom State, Nigeria in August, 2014. The plant was identified and authenticated by a taxonomist in Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPUU 573) was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

2.2 Extraction

The aerial parts were washed, shade-dried for 2 weeks and further reduced to powder using electric grinder. The powdered material was macerated in ethanol (50%) for 72 h. The liquid filtrate was concentrated and evaporated to dryness in vacuo 40°C using rotary evaporator. The liquid filtrate was concentrated and evaporated to dryness in vacuo 40°C using rotary evaporator. The dry extract was stored in a refrigerator at -4°C until use for the proposal experiment

2.3 Phytochemical Screening

Phytochemical screening of the crude extract was done using standard procedures and tests (Trease and Evans, 1989; Sofowora, 1993),

2.4 Animals

Albino rats (110 – 150 g) of both sexes were gotten from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

2.5 Determination of median lethal dose (LD₅₀)

The median lethal dose (LD₅₀) of the extract was determined in albino mice using intraperitoneal (i.p) route employing the method of Lorke (1983). This involved intraperitoneal treatment groups of three mice each with different doses of the extract (100 -1000 mg/kg). The animals were observed for manifestation toxicity signs such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each

group within 24 hours was recorded. The LD₅₀ was calculated as geometrical means of the maximum dose producing 0% and the minimum dose producing 100% mortality.

2.6 Animal treatment

Thirty-six (36) rats (male and female) were weighed and randomised into six groups of 6 rats each and treated as follows: Groups 1 animals were given only distilled water (0.2 ml/kg) and these were normal animals. Group 2 was pretreated with distilled water 0.2 ml/kg. Groups 3, 4 and 5 were respectively pretreated orally with 75, 150 and 225 mg/kg of *S. monostachyus* leaf extract daily for 8 days. Group 6 was pretreated with silymarin (100 mg/kg) (standard drug) for the same period of time. On the 8th day, Paracetamol, 2 g/kg, was administered to groups 2- 6.

Twenty-four hours post paracetamol administration, the animals were sacrificed under light diethyl ether vapour. Blood samples were collected by cardiac puncture and used immediately.

2.7 Hematological study

Animals were sacrificed under diethyl ether anesthesia and blood samples were collected from each rat by cardiac puncture into ethylene diamine tetra-acetic acid (EDTA) – coated sample bottles for analysis.

Hematological parameters which included red blood count (RBC), hemoglobin, (Hb), packed cell volume (PCV), platelet concentration (PLC) and total and differential white blood cell count (WBC) were determined. These were done using automatic hematological system (Sysmex Hematology – Coagulation system, Model MO-1000 I, Trans Asia, Japan).

2.8 Biochemical and histological evaluation of the hepatoprotective effect of the leaf extract in rats with paracetamol-induced Liver injury

Serum gotten from the blood of each rat sacrificed was stored at -20 °C until used for biochemical determinations such as total protein, albumin, aspartate aminotransferases (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, total and conjugated bilirubin. The determinations were carried out using Randox analytical kits according to standard procedures of manufacturer's protocols (Reitman and Frankel, 1957).

The livers of the animals were removed, weighed and a portion of each fixed in 10% formaldehyde for histological processes, while the other part was washed with ice cold 0.9% NaCl and homogenates were made in a ratio of 1 g of wet tissue to 9 ml of 1.25% KCl by using motor driven Teflon-pestle. The homogenates were centrifuged at 7000 rpm for 10 min at 4 °C and the supernatants were used for the assays of superoxide dismutase (SOD) (Marklund *et al*, 1974), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPX) (Lawrence and Burk, 1976), and reduced glutathione (GSH) (Ellman, 1959).

2.9 Biochemical and histological evaluation of the renoprotective effect of the leaf extract in rats with paracetamol-induced liver injury

The sera samples collected from the treated- rats were analyzed according to standard methods for effect of the extract on various Determination of kidney function parameters such as urea and creatinine as well as some ions like sodium, potassium and chloride were carried out in the sera of the treated rats. These analyses were done at Department of Chemical Pathology, University of Uyo Teaching Hospital, (UUTH), Uyo using various diagnostic kits. The kidneys surgically removed from the rats were weighed and a portion of each fixed in 10% formaldehyde, processed, sectioned and stained with Heamatoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo.

The other part of each kidney removed was dissected free from the surrounding fat and connective tissue. Each kidney was longitudinally sectioned, and renal cortex was separated and kept at -8°C . Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 5000rpm for 10 min at 4°C . The resulting supernatant was used for the determination of - malondialdehyde (MDA) content (Wilbur *et al*, 1949; Esterbauer and Cheeseman, 1990), reduced glutathione (GSH) levels and antioxidant enzyme levels such as superoxide dismutase (SOD), and catalase (CAT) activities using colorimetric assay.

2.10 Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using ANOVA (One - way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5% level of significance i.e. $p \leq 0.05$.

3. Results

Alkaloids, cardiac glycosides, tannins, saponins, terpenes and flavonoids were found to be present in the ethanol extract of the aerial parts of *Solenostemon monostachyus*

The median lethal dose (LD_{50}) was determined to be 748.331 mg/kg and excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma were observed before death of the animals.

Effect of leaf extract on haematological parameters of rats

Treatment of rats with paracetamol (2 g/kg) did not cause any significant effect ($p > 0.05$) on RBC, WBC PCV, and Hemoglobin concentration when compared to normal control. Administration of paracetamol to rats caused significant ($p < 0.001$) reductions in percentages of neutrophils ($15.75 \pm 0.13\%$) and monocytes ($1.50 \pm 0.18\%$) when compared to normal control (**Table 1**). However, pre-treatment of rats with *S. monostachyus* leaf extract (75 -225 mg/kg) was found to significantly ($p < 0.001$) increased neutrophils and monocytes percentages to 37.0 ± 1.30 and 7.25 ± 0.20 respectively when compared to the untreated group. Also, the

significantly ($p < 0.05 - 0.001$) increased lymphocytes percentage (92.75 ± 2.83) due to paracetamol administration was significantly reduced in the extract and silymarin - treated rats to $58.60 \pm 5.35\%$ and $63.50 \pm 7.08\%$ respectively ($p < 0.01$).

The increase in platelet counts induced by paracetamol (833.0 ± 17.51) was not significant ($p > 0.05$) when compared to control (736.2 ± 55.70). However, the extract administration produced a significant ($p < 0.01$) increase in platelet count (935.2 ± 56.29) when compared to the control. Silymarin did not affect platelet count significantly ($p > 0.05$) when compared to control.

The percentages of eosinophils and basophils were not affected by paracetamol administration but higher doses of the extract (150 and 225 mg/kg) were found to significantly ($0.05 - 0.001$) increase the percentages of eosinophil to 2.75 ± 1.10 and 2.33 ± 1.15 respectively when compared to control (**Table 1**).

Effect of leaf extract on liver and kidney weights

The liver and kidney weights of paracetamol-treated rats were significantly ($p < 0.001$) increased when compared to that of the control group. However, pre-treatment with the leaf extract and silymarin was found to significantly ($p < 0.01 - 0.001$) reduced the weights of these organs (**Table 2**).

Effect of leaf extract on liver function parameters in rats with paracetamol-induced Liver injury

Administration of paracetamol (2 g/kg) to rats was found to cause significant ($p < 0.001$) increases in liver enzymes levels; AST, ALT and ALP. 171.25 ± 1.23 , 72.62 ± 5.52 , and 155.0 ± 4.52 IU/L were respectively recorded for AST, ALT and ALP when compared to control. Total cholesterol, total and conjugated bilirubin were also elevated to 3.42 ± 0.11 mMol/L, 16.0 ± 0.50 and 9.48 ± 0.52 mg/dl respectively. Decreases in total protein ($3.60.0 \pm 0.14$ g/dl) and albumin (2.01 ± 0.16 g/dl) levels were also observed with paracetamol.

The middle dose (150 mg/kg) of the extract was observed to cause significant ($p < 0.01 - 0.001$) decreases in the levels of ALT and AST to 135.2 ± 3.20 and 37.24 ± 3.44 IU/L respectively when compared to the untreated group. Silymarin was also observed to caused significant ($p < 0.01 - 0.001$) decreases in the levels of ALT and AST to 135.5 ± 6.80 and 35.54 ± 9.60 IU/L respectively when compared to the untreated group.

The low and highest doses of the extract (75 and 225 mg/kg) could not reverse the elevated ALT and AST levels caused by paracetamol. Pretreatment of rats with leaf extract of *S. monostachyus* (75-225 mg/kg) caused a dose-dependent significant ($p < 0.01 - 0.001$) decreases in the levels of ALP, total cholesterol, total and conjugated bilirubin when compared with the paracetamol group. Total protein and albumin levels were significantly ($p < 0.05 - 0.001$) increased dose-dependently in the groups pre-treated with the leaf extract when compared to the paracetamol group (**Table 3**).

Table 1: Effect of treatment with ethanol extract of *Solenostemon monostachyus* on the haematological parameters of rats with paracetamol-induced hepato-nephrotoxicity.

| Treatment Dose (mg/kg) | RBC (X 10 ¹² /l) | PCV (%) | Hb (g/dl) | WBC (X 10 ⁹ /l) | Neutrophils (%) | Lymphocytes (%) | Monocytes (%) | Eosinophils (%) | Basophils (%) | Platelet |
|--------------------------|-----------------------------|------------|------------|----------------------------|--------------------------|-------------------------|--------------------------|--------------------------|---------------|--------------------------|
| Normal control | 6.92±0.43 | 47.3±1.33 | 9.98±0.45 | 7.54±1.14 | 29.80±1.62 | 70.70±3.88 | 2.50±0.12 | 0.75±0.01 | 0.75±0.20 | 736.2±55.70 |
| PCM +Dist. water | 6.92±0.53 | 50.57±1.49 | 8.07±0.09 | 9.92±2.58 | 15.75±0.13 ^b | 92.75±2.83 ^a | 1.50±0.18 ^c | 0.00±0.00 | 0.50±0.01 | 833.0±17.51 |
| SM. 75mg/kg + PCM | 7.35±0.30 | 47.5±1.34 | 13.90±0.48 | 12.62±1.19 | 37.0±1.30 ^f | 58.60±5.38 ^f | 3.00±0.14 ^f | 1.00±0.55 | 0.50±0.01 | 820.7±10.33 |
| SM. 150mg/kg+ PCM | 6.95±0.17 | 49.1±0.70 | 12.51±0.25 | 13.12±1.07 | 25.0±2.88 | 65.82±3.80 ^e | 4.66±0.10 ^{c,f} | 2.33±1.15 ^{c,f} | 0.00±0.00 | 935.2±56.29 ^a |
| SM 225mg/kg + PCM | 7.37±1.40 | 50.6±0.65 | 13.38±0.40 | 12.38±1.88 | 24.0±3.18 | 65.97±4.60 ^e | 7.25±0.20 ^{c,f} | 2.75±1.10 ^{c,f} | 0.50±0.01 | 930.2±36.29 ^a |
| Silymarin 100mg/kg + PCM | 7.14±0.60 | 51.46±3.70 | 11.16±0.50 | 11.50±2.22 | 41.0±2.49 ^{a,f} | 63.50±7.08 ^e | 4.20±0.12 ^{c,f} | 0.80±0.11 | 1.00±0.11 | 871.8±64.56 |

Data were expressed as mean ± SEM. Significant at ^ap< 0.05, ^bp< 0.01, ^cp< 0.001 when compared to control. ^dp< 0.05, ^ep< 0.01, ^fp< 0.001 when compared to paracetamol . n = 6.

Table 3: Effect of *Solenostemon monostachyus* on liver function of PCM -induced liver injury in rats

| Parameters/ Treatment | Total protein (g/dl) | Albumin (g/dl) | Total bilirubin (mg/dl) | Conjugated bilirubin (mg/dl) | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | Total cholesterol (Mmol/L) | Liver weight (g) |
|--------------------------|------------------------|------------------------|-------------------------|------------------------------|---------------------------|-------------------------|-------------------------|----------------------------|------------------------|
| Normal control | 4.58± 0.16 | 2.82±0.12 | 12.2±0.50 | 5.78±0.58 | 123.2±1.88 | 37.20±1.88 | 105.2±4.32 | 2.28± 0.22 | 7.75±1.11 |
| PCM +Dist. water | 3.60±0.14 ^c | 2.01±0.16 ^c | 16.0±0.50 ^c | 9.48±0.52 ^c | 171.25± 1.23 ^c | 72.62±5.52 ^c | 155.0±4.52 ^c | 3.42±0.11 ^c | 9.37±0.53 ^c |
| SM. 75mg/kg + PCM | 4.52±0.12 ^e | 2.90±0.18 ^d | 15.0± 0.34 ^a | 8.22±0.44 ^b | 156.25±9.81 ^b | 53.75±9.98 | 118.6±6.13 ^f | 2.25±0.08 ^f | 8.18±0.13 ^f |
| SM. 150mg/kg+ PCM | 4.48±0.15 ^e | 2.90±0.12 ^e | 12.5±0.94 ^e | 6.10±0.77 ^e | 135.2± 3.20 ^f | 37.24±3.44 ^e | 113.7±4.52 ^f | 2.34±0.11 ^f | 8.11±0.20 ^f |
| SM.225mg/kg+ PCM | 4.25±0.20 ^e | 2.68±0.15 ^f | 13.0±0.27 ^a | 8.42±0.39 ^b | 164.75±4.83 ^c | 68.23±2.38 ^a | 123.7±3.45 ^e | 2.60±0.04 ^f | 8.03±0.16 ^f |
| Silymarin 100mg/kg + PCM | 4.62±0.15 ^f | 2.90±0.13 ^f | 12.5±0.64 ^e | 6.46±0.23 ^e | 135.5±6.80 ^f | 35.54±9.60 ^f | 98.75±8.67 ^f | 2.10±0.04 ^f | 7.89±0.18 ^f |

Data were expressed as mean ± SEM. Significant at ^ap< 0.05, ^bp< 0.01, ^cp< 0.001 when compared to control.^dp< 0.05, ^ep< 0.01, ^f< 0.001 when compared to paracetamol . n = 6.

Table 2: Effect of *Solenostemon monostachyus* extract on Liver and Kidney weights in paracetamol-induced kidney injury in rats.

| Treatment | Kidney weight (g) | Liver weight (g) |
|--------------------------|------------------------|------------------------|
| Normal control | 0.72±0.01 | 7.75±1.11 |
| PCM +Dist. Water | 1.21±0.06 ^c | 9.37±0.53 ^c |
| SM. 75mg/kg + PCM | 0.66±0.02 ^f | 8.18±0.13 ^f |
| SM. 150mg/kg+ PCM | 0.70±0.03 ^f | 8.11±0.20 ^f |
| SM.225mg/kg+ PCM | 0.67±0.02 ^f | 7.99±0.16 ^f |
| Silymarin 100mg/kg + PCM | 0.72±0.03 ^f | 7.89±0.18 ^f |

Data were expressed as mean ± SEM. Significant at ^a p< 0.05, ^b p< 0.01, ^c p< 0.001 when compared to control. ^d p< 0.05, ^e p< 0.01, ^f p< 0.001 when compared to paracetamol. n = 6.

Effect of the extract on kidney function parameters in rats with paracetamol-induced kidney injury

Treatment of rats with paracetamol (2 g/kg) was observed to caused significant (p<0.001) increases in serum urea and creatinine levels to 8.48±0.35mMol/L and 168.4 ±3.12 mMol/L respectively when compared to control. Pre-treatment of the rats with leaf extract of *S. monostachyus* (75 – 225 mg/kg) significantly (p<0.01) reduced the elevated levels of serum urea and creatinine in a dose-dependent fashion to 5.44±0.38 mMol/L and 109.8±13.58 mMol/L respectively when compared to the untreated group. Silymarin also caused significant (p<0.01) reduction in serum urea and creatinine levels to 7.36±1.68 mMol/L and 102.75±12.33 mMol/L respectively when compared to control. However, electrolytes (K⁺, Na⁺, Cl⁻ and HCO₃⁻) levels of rats were not affected by paracetamol administration as well as pre-treatment with the extract (Table 4).

Effect of leaf extract on liver and kidney antioxidant enzymes

Antioxidant enzymes and indices such as SOD, catalase, GPx and GSH level in liver tissue were observed to be significantly (p<0.001) reduced by paracetamol treatment to 9.87±0.30, 27.85± 0.96, 12.23±0.45, and 0.12±0.01 U/mg of protein respectively when compared with control group. These reductions were reversed by pre-treatment with leaf extract of *S. monostachyus* (75-

225 mg/kg) which resulted in a significant (p<0.05 – 0.001) increase in the activities of SOD, catalase, GPx and GSH level to 16.16± 0.18, 46.38±2.19, 21.39±0.18 and 0.27±0.02 U/mg of protein respectively at the highest dose of the extract (225 mg/kg). Similar increases were observed in silymarin-treated rats with values of 19.02±0.15, 50.47± 1.92, 22.11±0.81 and 0.31±0.01 U/mg of protein respectively for SOD, catalase, GPx and GSH (Table 5).

Administration of paracetamol to rats caused significant reduction in levels of SOD, catalase and GSH with values of 7.25 ± 0.21, 22.56± 2.10 and 0.10±0.01 U/mg of protein respectively when compared to control (p<0.001). Pre-treatment with leaf extract of *S. monostachyus* (75–225 mg/kg) resulted in significant (p<0.05–0.001) increases in the activities of SOD, catalase and GSH levels in kidney tissue with values of 17.44± 0.25, 34.22±1.06 and 0.22±0.01U/mg of protein respectively. Concentration of MDA which was significantly (p<0.001) elevated to 72.29±2.98 U/mg of protein in kidney tissue by paracetamol treatment was also reduced significantly (p<0.001) by extract pretreatment to 53.97±2.18 U/mg of protein. Silymarin treated animals also showed significant (p<0.001) increases in antioxidant enzymes with values of 19.70 ±0.11, 38.02± 2.16 and 0.30±0.01U/mg of protein for SOD, catalase and GSH respectively and decrease in the level of MDA (48.56±1.86 U/mg of protein) compared to paracetamol treated rats (Table 6).

Table 4: Effect of ethanol extract of *Solenostemon monostachyus* on some kidney function parameters of rat

| Parameters/ Treatment | Na ⁺ (mmol/l) | K ⁺ (mmol/l) | Cl ⁻ (mmol/l) | HCO ₃ ⁻ (mmol/l) | UREA (mmol/l) | Creatinine (mmol/l) | Kidney weight (g) |
|------------------------------|-----------------------------|----------------------------|-----------------------------|---|--------------------------|---------------------------|------------------------|
| Normal control | 140.4±0.40 | 9.56±0.39 | 102.6±0.92 | 27.6±0.50 | 5.72±0.72 | 106.2±3.12 | 0.72±0.01 |
| PCM +Dist. Water | 143.0±0.83 | 8.72±0.33 | 102.6±0.66 | 28.8±0.73 | 8.48±0.35 ^c | 168.4 ±3.12 ^c | 1.21±0.06 ^c |
| SM. 75mg/kg + PCM | 142.7±0.94 | 8.12±0.12 | 102.7±1.70 | 28.25±0.85 | 6.86±0.70 ^{c,f} | 133.2±9.51 ^{a,d} | 0.66±0.02 ^f |
| SM. 150mg/kg+ PCM | 142.6 ± 0.92 | 9.06±0.82 | 104.4±0.67 | 28.00± 0.66 | 5.82±0.62 ^f | 107.4±2.33 ^f | 0.70±0.03 ^f |
| SM.225mg/kg+ PCM | 132.8 ± 6.71 | 8.52±0.34 | 104.1±2.11 | 26.80± 0.86 | 5.44±0.38 ^f | 109.8±13.58 ^f | 0.67±0.02 ^f |
| Silymarin 100mg/ kg + PCM | 139.2±1.10 | 8.32±0.76 | 101.8±0.20 | 27.25± 0.25 | 7.36±1.68 ^{c,e} | 102.75±12.33 ^f | 0.72±0.03 ^f |

Data were expressed as mean ± SEM. significant at ^a p< 0.05, ^b p< 0.01, ^c p< 0.001 when compared to control. ^d p< 0.05, ^e p< 0.01, ^f p< 0.001 when compared to paracetamol. n = 6.

Table 5: Effect of *Solenostemon monostachyus* leaf extract on liver antioxidant enzymes

| Treatment | SOD (U/mg of protein) | CAT (U/mg of protein) | GPx (U/mg of protein) | GSH (µg/mg of protein) |
|---------------------------|----------------------------|----------------------------|----------------------------|---------------------------|
| Normal control | 20.14 ± 0.22 | 58.34 ± 1.14 | 23.27 ± 0.48 | 0.35 ± 0.01 |
| PCM +Dist. Water | 9.87 ± 0.30 ^c | 27.85 ± 0.96 ^c | 12.23 ± 0.45 ^c | 0.12 ± 0.01 ^c |
| SM. 75mg/kg + PCM | 12.38 ± 0.11 ^{cf} | 33.32 ± 0.99 ^c | 17.99 ± 0.88 ^{cf} | 0.14 ± 0.01 ^c |
| SM 150mg/kg+ PCM | 15.17 ± 0.14 ^{cf} | 39.16 ± 1.12 ^{cf} | 19.73 ± 0.46 ^{cf} | 0.24 ± 0.01 ^{bf} |
| SM. 225mg/kg+ PCM | 16.16 ± 0.18 ^{cf} | 46.38 ± 2.19 ^{cf} | 21.39 ± 0.18 ^f | 0.27 ± 0.02 ^f |
| Silymarin 100 mg/kg + PCM | 19.02 ± 0.15 ^{bf} | 50.47 ± 1.92 ^{bf} | 22.11 ± 0.81 ^f | 0.31 ± 0.01 ^f |

Data were expressed as mean ± SEM. significant at ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 when compared to control. ^d p < 0.05, ^e p < 0.01, ^f p < 0.001 when compared to paracetamol. n = 6.

Table 6: Effect of *Solenostemon monostachyus* leaf extract on kidney antioxidant enzymes in paracetamol-induced kidney injury in rats.

| Parameters/ Treatment | SOD (U/mg of protein) | CAT (U/mg of protein) | MDA (U/mg of protein) | GSH (µg/mg of protein) |
|---------------------------|----------------------------|----------------------------|----------------------------|---------------------------|
| Normal control | 20.10 ± 0.16 | 45.27 ± 0.48 | 40.04 ± 1.28 | 0.38 ± 0.01 |
| PCM +Dist. Water | 7.25 ± 0.21 ^c | 22.56 ± 2.10 ^c | 72.29 ± 2.98 ^c | 0.10 ± 0.01 ^c |
| SM. 75mg/kg + PCM | 10.27 ± 0.36 ^{cd} | 25.48 ± 0.17 ^c | 64.28 ± 1.20 ^c | 0.15 ± 0.01 ^{cd} |
| SM 150mg/kg+ PCM | 15.42 ± 0.20 ^{cf} | 32.56 ± 2.02 ^{bf} | 56.42 ± 1.46 ^{cf} | 0.20 ± 0.01 ^{cf} |
| SM. 225mg/kg+ PCM | 17.44 ± 0.25 ^{cf} | 34.22 ± 1.06 ^{cf} | 53.97 ± 2.18 ^{cf} | 0.22 ± 0.01 ^{cf} |
| Silymarin 100 mg/kg + PCM | 19.70 ± 0.11 ^f | 38.02 ± 2.16 ^{af} | 48.56 ± 1.86 ^{af} | 0.30 ± 0.01 ^{af} |

Data were expressed as mean ± SEM. significant at ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 when compared to control. ^d p < 0.05, ^e p < 0.01, ^f p < 0.001 when compared to paracetamol. n = 6.

Effect of leaf extract on liver histology in rats with paracetamol-induced liver injury

Histopathological examination of liver sections of control group revealed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (**Figure 1A**). Toxicant (paracetamol) group demonstrated severely distorted architecture, widened spaces between plates of hepatocytes which contain haemorrhage, moderate portal inflammation, moderate congested sinusoid and focal necrosis (**Figure 1B**). The liver sections of the rats treated with leaf extract of *S. monostachyus* (75 - 150 mg/kg) demonstrated signs of protection as was evident by mild inflammation, reduction of vascular congestion and degeneration, cellular degeneration, necrosis and vacuoles (**Figure 1D** and **1E**), while the liver sections of the pretreated rats with *S. monostachyus* (225 mg/kg) were observed to show little or no protection with mild portal inflammation, moderate congested central veins/sinusoids and severe necrosis (**Figure 1F**). Silymarin (100 mg/kg) treated rats showed significant protection and absence of necrosis and inflammation (**Figure 1C**).

Effect of leaf extract on the kidney histology in rats with paracetamol-induced kidney injury

The histological pattern of normal kidney showed preserved architecture with normal glomeruli normal tubules and normal interstitial and Bowman's capsule (**Figure 2A**). Treatments with paracetamol showed severely distorted architecture with moderately

damaged glomeruli and tubules, congested to thrombosed interstitial blood vessels with inflammation, interfollicular stroma containing severe congested blood vessels and areas of haemorrhage (**Figure 2B**). The rats treated with leaf extract of *S. monostachyus* (75-225 mg/kg) showed preserved architecture with normal glomeruli, normal tubules, slight inflammation and congested to thrombosed interstitial blood vessels (**Figure 2D- 2F**). Rats treated with silymarin showed preserved architecture with normal glomeruli, normal tubules and congested interstitial blood vessels (**Figure 2C**).

4.0 Discussion

In this study, the ethanol leaf extract of *S. monostachyus* was evaluated for hepatoprotective and renoprotective activities against paracetamol-induced hepatonephrotoxicity in rats. Liver and kidney function tests, liver and kidney antioxidant enzymes levels and histological studies were used to assess hepatoprotective and renoprotective properties. The results of this study indicated that paracetamol administration caused liver and kidney toxicities as observed in alteration of various liver and kidney parameters such as elevations of liver enzymes, urea and creatinine levels as well as other parameters. Paracetamol was found to cause various degrees of injuries to the organs. Considerable decreases in tissue GSH level with reduction in activities of liver antioxidative enzymes such as SOD and CAT and elevation of MDA level were equally observed.

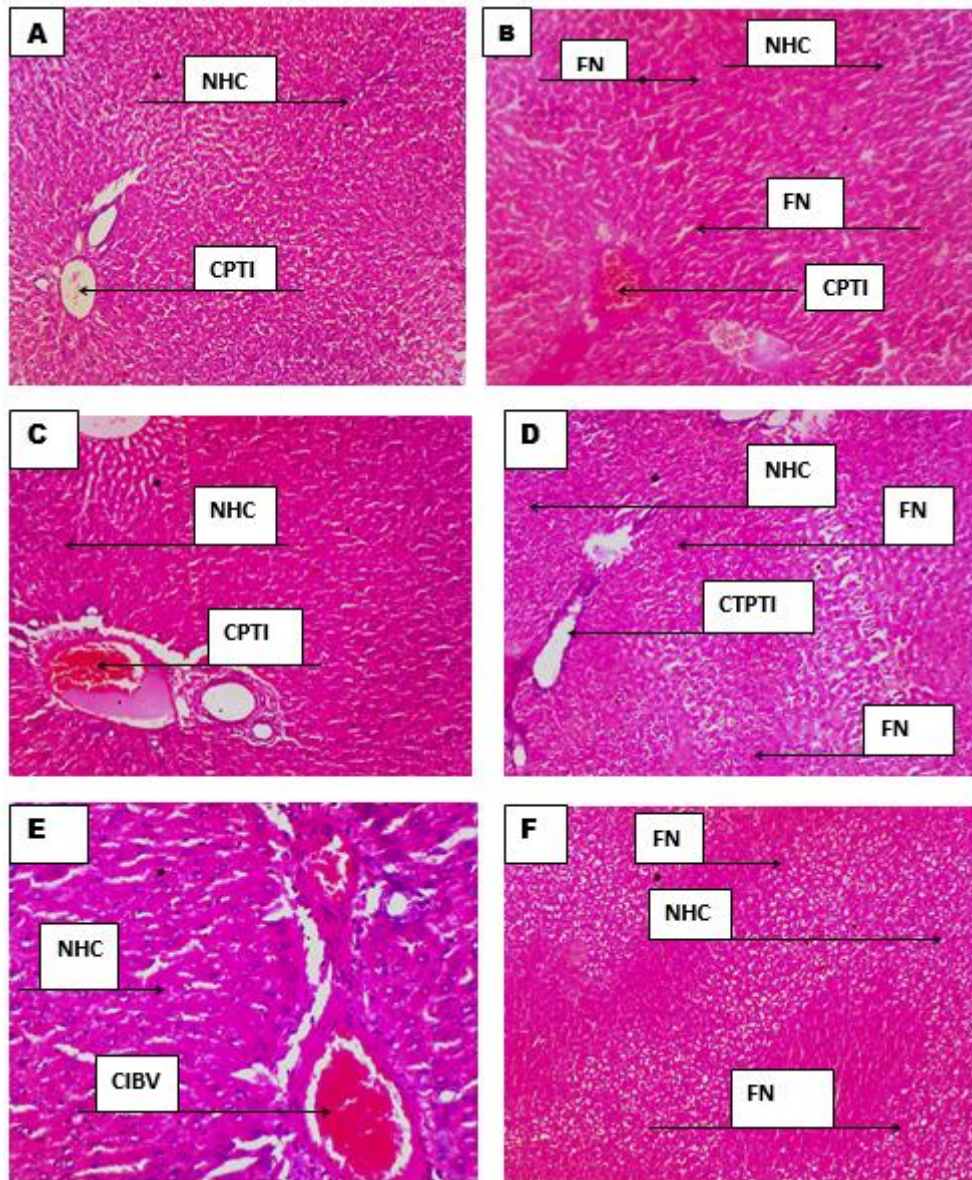


Figure 1. Photomicrographs of livers of rats treated with distilled water (A), Paracetamol 2g/kg (B), Sylimarin (C), *S. monostachyus* (75mg/kg) (D), *S. monostachyus* (150mg/kg) (E), *S. monostachyus* (225mg/kg) (F). Normal hepatocytes (NHC), focal necrosis (FN), congested portal tract with inflammation (CPTI), congested interstitial blood vessels (CIBV) H & E Magnification X 10.

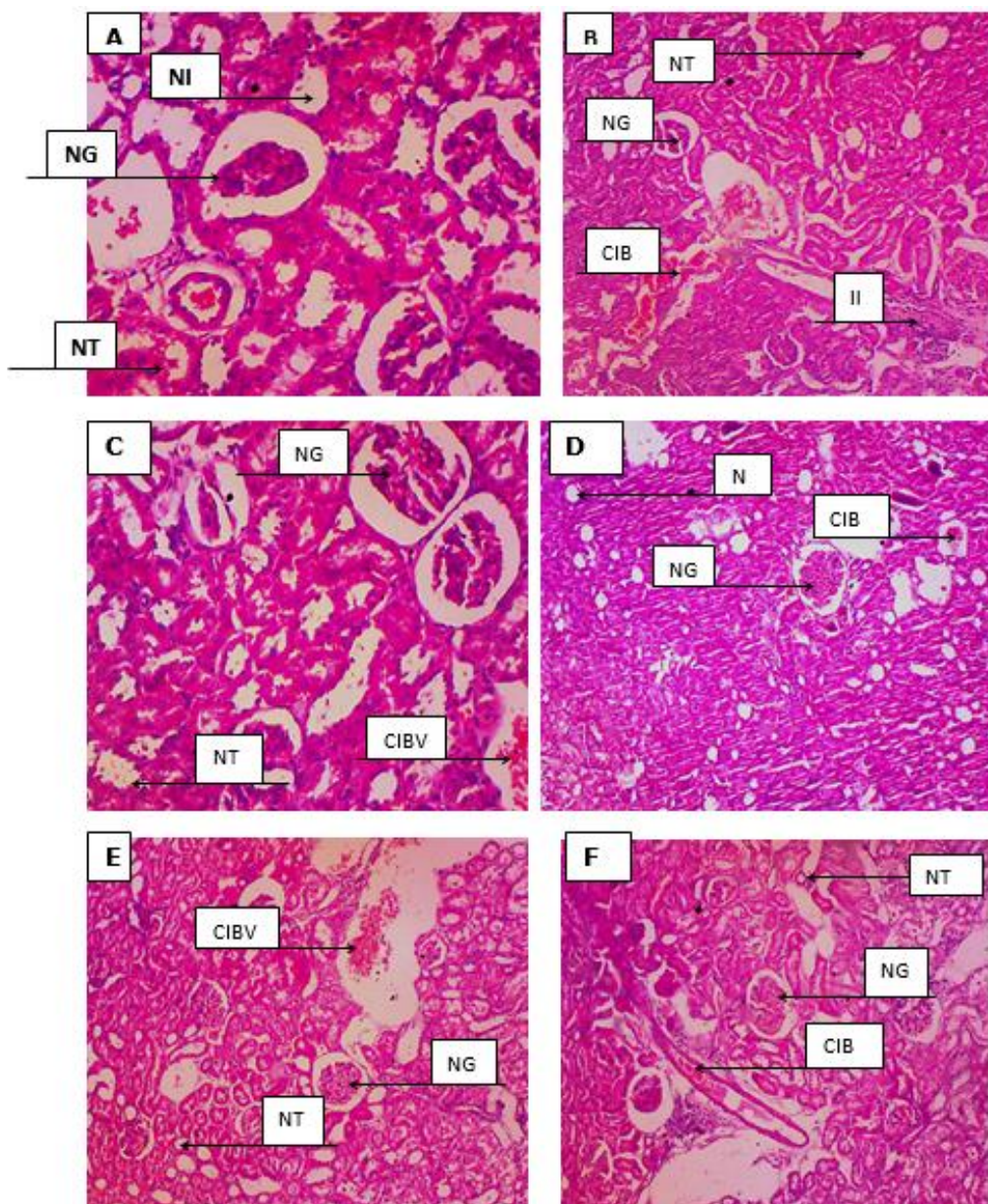


Figure 2. Photomicrographs of kidneys of rats treated with distilled water (A), Paracetamol 2g/kg (B), Sylmarin (C), *S. monostachyus* (75mg/kg) (D), *S. monostachyus* (150 mg/kg) (E), *S. monostachyus* (225mg/kg) (F). Normal glomeruli (NG), normal tubules (NT), Inflammation (II), Normal interstitial (NI), Congested interstitial blood vessels (CIBV) H & E Magnification X 10.

Paracetamol is often used in the treatment of fever and pains. Unfortunately, it produces acute toxic effect at high doses which leads to liver damage. The drug is metabolised to a toxic electrophile, *N*-acetyl *p*-benzoquinone imine (NAPQI), which affect tissue components and alters the homeostasis of calcium (Lin et al, 1997). This further generate reactive species which leads to depletion of protective physiological moieties (glutathione and α -tocopherol, etc.), causing injuries to the macromolecules in vital biomembranes (Aldridge, 1981; Gilani et al, 2005).

Liver function parameters such as serum ALT, AST, ALP, bilirubin (total and direct), total cholesterol, total protein and albumin are used to establish liver dysfunction (Manokaran et al, 2008). During

hepatopathy, liver enzymes and molecules leak into the blood stream which indicates liver damage (Nkosi et al, 2005). The elevated levels of serum ALT, AST, ALP, total bilirubin (total and direct), and total cholesterol as well as reductions in levels of total protein and albumin as observed in paracetamol group in our study suggest injury to hepatic cells and liver damage due to paracetamol. The extracts' ability to reverse the elevated serum enzymes levels induced by paracetamol indicates protective potentials maybe by preventing the effect of the free radicals generated by paracetamol on liver tissue through antioxidant activities of its components. Synthetic ability of the liver was found to be compromised following paracetamol treatment as serum levels of total protein and albumin were observed to be reduced. This is likely to result from

reduction in the number of hepatocytes and further revealed the severity of hepatopathy. These reductions in total protein and albumin levels were reversed by extract pretreatment, indicating hepatoprotective activity by the extract.

In this study, bilirubin level elevated by paracetamol was found to be significantly reduced by extract pretreatment. Estimation of serum bilirubin is a useful index to assess hepatic function, and any abnormal increase in the levels of serum bilirubin indicates hepatobiliary disease and severe disturbance of hepatocellular function (Martin and Friedman, 1992). Reduction in serum bilirubin level following extract pretreatment further suggest the protective potential of the extract.

Paracetamol-induced toxicity in rats is likely affects membrane structure and function as well as lipids metabolism in the liver as suggested by the increased cholesterol levels of rats. This effect was reduced by the protective activity of the leaf extract which reduced the increased level of total cholesterol.

SOD and CAT are important enzymes in the enzymatic antioxidant defense system (Curtis et al, 1972) and decreases in their activities may lead to a number of injurious effects. Pre-treatment with *S. monostachyus* leaf extract was observed to significantly ($p < 0.05$) increased hepatic SOD and CAT activities compared to control paracetamol treated group. This suggest that *S. monostachyus* possess the ability to scavenge reactive free radicals, thereby reducing their effects on the tissues in addition to improving activity of hepatic antioxidant system. This is further support by the observation that pretreatment with *S. monostachyus* leaf extract significantly increased the level of GPx and GSH in a dose dependent manner strongly portraying its ability to scavenge these free radicals.

Haematological parameters such as RBC, Hb, PCV, WBC and basophil and eosinophil percentages were not affect by paracetamol treatment in this study, except reductions in the percentages of neutrophils and monocytes of paracetamol-treated rats. Pretreatment with *S. monostachyus* leaf extract caused significant ($p < 0.05 - 0.001$) increases against reductions induced by paracetamol and considerable reduction of lymphocytes though in a non-dose dependent fashion.

The histological findings corroborate that of the biochemical results laying credence to the hepatoprotective potentials of the extract and validating its use as antidote.

Kidneys are responsible for the excretion of xenobiotics, pollutants, toxins and are exposed to high quantities of free radicals which contribute to high oxidative stress. This contributes majorly to the pathogenesis of kidney damage. The ethanol leaf extract of *S. monostachyus* was also evaluated for nephroprotective activity against paracetamol-induced nephrotoxicity in rats. p-amino phenol(PAP), a metabolite of paracetamol is implicated in the pathogenesis of paracetamol induced renal damage (Carpenter and Mudge, 1981; Mugford and Tarloff, 1997). Kidney function parameters such as blood urea, serum creatinine, electrolytes and tissue GSH levels, tissue SOD and CAT activities and MDA level

were used to assess nephroprotective activity. Significant decrease in tissue level of GSH level with increased MDA level a product of lipid peroxidation were observed in this study following paracetamol treatment. Kidney antioxidative enzymes like SOD and CAT activities were also reduced. Serum urea and creatinine level were also observed to be elevated and varying degrees of histological lesions were observed as well. Pretreatment of the animals with leaf extract of *S. monostachyus* was found to reverse these effects on the kidney suggesting nephroprotective potentials of the extract.

The nephro protective property of the extract is further confirmed by significant improvement of the kidney architecture by reversing the glomerular congestion, interstium with inflammatory cells, tubular necrosis, peritubular necrosis and basement degeneration over paracetamol administered group.

The leaf extract and fractions have been reported to exhibit strong antioxidant (Datte et al, 2010; N'guessan et al, 2011; Okoko and Ere, 2012) activity. The leaf essential oil of *S. monostachyus* has been reported to contain; β -pinene, oct-1-en-3-ol, β -caryophyllene, octan-3-ol and (*E,E*)- α -farnesene (Mvé-Mba et al, 1994) and flavonoids, coumarin and polyphenol (Datte et al, 2010; N'guessan et al, 2011) have also been isolated from the leaf of the plant. These compounds are potential antioxidant compounds and could possibly account for the hepatoprotective and nephroprotective properties of this plant. The strong antioxidant activity of this extract and constituents explains the significant hepatoprotective and nephroprotective activities of the leaf extract and may as well explain the mechanism of action of the observed hepatoprotective and nephroprotective activities of *S. monostachyus*.

5.0 Conclusion

The findings of this study further support the liver and renal protective potentials of leaf of *S. monostachyus* which can be attributed to the antioxidant properties of its constituents. Hence, the leaf of *S. monostachyus* posses' hepato and nephroprotective activities against paracetamol- induced liver and kidney injuries.

Conflict of Interest declaration

The authors declare no conflict of interest.

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