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RESEARCH ARTICLE

Effect of *Spathodea campanulata* Ethanol Leaf Extract on Hematology and Liver Function of *Salmonella*-infected and Paracetamol-induced Swiss Albino Mice

Fred. Coolborn AKHARAIYI[*°], Arthur Chinedu OKAFOR[**]

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SUMMARY

Herbal remedies for healing is basically on the existing traditional methods, which is different from one tradition to the other. Liver performs useful functions that maintain health in humans but it can be affected to become malfunction if not guided or protected against some chemical substances contained in some foods, hard drugs and drinks. Effect on hematology and hepatoprotective activity of Spathodea campanulata ethanol leaf extract was studied using an animal model. Group I mice served as the positive control, group II mice as negative control, and groups III – XII mice as satellite groups which were treated with 200, 400, 800, 1000, and 2000 mg/kg of extract after respective Salmonella typhi infection and paracetamol inducement. Overdose of mice with paracetamol caused changes in the mice's physiology status. In hematology parameters of mice, red blood cell mean count was higher in the negative control (7.6±70.92 million/mm^3) than the positive control (4.36±0.12 million/ mm^3) and lower white blood cells mean count of 3.50±0.18 thousand/mm^3 in the negative control than positive control with a value of 9.62±0.39 thousand/mm^3. However, in biochemical evaluation, albumin (2.21±0.60 mg/dL) and bilirubin (2.11±0.63 mg/dL) were higher in the positive control than negative control with values of 4.90±0.11 and 1.08±0.10 mg/dL, respectively. These abnormalities in the mice's physiological status were reversed on treatment with extract concentrations of 200 to 2000 mg/mL for five days. S. campanulata ethanol leaf extract can be used as traditional medicine for the treatment of liver diseases.

Key Words: *Liver function, Spathodea campanulata, paracetamol, Salmonella typhi.*

Spathodea campanulata Yaprağı Etanol Ekstresinin Salmonella ile Enfekte Olan ve Parasetamol ile İndüklenmiş Swiss Albino Farelerin Hematolojisi ve Karaciğer Fonksiyonu Üzerine Etkisi

ÖZ

Tedavi için bitkilerin kullanımı, temelde bir gelenekten diğerine farklı olmakla birlikte mevcut geleneksel yöntemlere dayanmaktadır. Karaciğer, insan sağlığını koruyan fonksiyonlarda görev alır, ancak bazı yiyeceklerde, içeceklerde ve ilaçlarda bulunan çeşitli kimyasal maddelere karşı karaciğerde hasar oluşabilir. Spathodea campanulata yapraklarının etanol ekstresinin hematoloji ve hepatoprotektif aktivitesi üzerindeki etkisi hayvan modeli kullanılarak incelenmiştir. Grup I fareler pozitif kontrol, grup II fareler negatif kontrol olarak ve grup III - XII fareler uydu grupları olarak kullanılmış, Salmonella typhi enfeksiyonu ve parasetamol indüksiyonundan sonra 200, 400, 800, 1000 ve 2000 mg/kg dozda ekstre ile uygulama yapılmıştır. Parasetamolün aşırı dozu, farelerin fizyolojik durumunda değişikliklere neden olmuştur. Farelerin hematoloji parametreleri incelendiğinde, ortalama kırmızı kan hücresi sayısı negatif kontrolde (7,6 ± 70,92 milyon/mm^3) pozitif kontrolden (4,36 ± 0,12 milyon/ mm^3) daha yüksek ve ortalama beyaz kan hücresi sayısı negatif kontrolde (3,50 ± 0,18 bin/mm^3) pozitif kontrole göre (9,62 ± 0,39 bin/mm^3) daha düşük gözlenmiştir. Ancak biyokimyasal değerlendirmede albümin (2,21 ± 0,60 mg/dL) ve bilirubin (2,11 ± 0,63 mg/dL) negatif kontrole göre sırasıyla 4,90 ± 0,11 ve 1,08 ± 0,10 mg/dL değerlerinde daha yüksek bulunmuştur. Farelerdeki fizyolojik değişikliklerdeki bu anormallikler, beş gün süreyle 200-2000 mg/mL konsantrasyonlarda ekstre uygulaması ile tersine çevrilmiştir. S. campanulata yapraklarının etanollü ekstresi, karaciğer hastalıklarının tedavisi için geleneksel ilaç olarak kullanılabilir.

Anahtar Kelimeler: *Karaciğer fonksiyonu, Spathodea campanulata, parasetamol, Salmonella typhi.*

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INTRODUCTION

The majority of urban and rural duelers prioritize in managing diseases with the accessible and available herbal remedies within their localities. This type of practice is adaptable because herbal plants are effective in the management of diseases. It is certain that all over the world, there exist plants that produce defensive secondary metabolites against microbial infections. As reported by World Health Organization (WHO), herbalism is of high percentage in continents like Asia and Africa, where herbal medicines serve primary health care (Khan, 2016). The traditional medicine relied upon by a large population of the world is derived from plant materials (Deshwal, 2011; Arbab, 2016).

The liver is an organ that performs vital functions for the maintenance of health in humans. However, the food we consume and some environmental hazards do affect the liver to relieve or reduce its operations. Outside these, some chemicals such as anti-tuberculosis drugs, paracetamol, carbon tetrachloride (CCl_4), and alcohol have been found injurious to the liver in high dosage (Arbab, 2016). The setting in of such disorders in liver need medical attention as well as protection to avoid infection. Hepatic diseases are recognized by international public health, and there has not been a reliable and suitable drug for their cure. Based on this fact, the need to search and develop effective alternative drugs for liver protection requires urgent attention. For the fact that liver malfunction will reflect vividly in the biochemical physiology and hematological profiles, this study, therefore, was focused on how the plant remedy can be employed to manage or cure the liver infection.

Spathodea campanulata P. Beauv species belong to the Bignoniaceae family. Phytochemicals such as tannins, sterols, vanilic acid, verminoside, triterpenoids, ferulic acid, sterol spathodol, chlorogenic acid, maldivin, quercetin, caffeic acid, flavonoids, steroids, alkaloids, phenol, terpenoids, saponins, anthraquinones, phlobatannins have been identified from the plant (Ngouela, 2001; Akharaiyi, 2015). Report by Adriana et al. (2007) stated that preparations from stem bark and leaves of the plant have value in traditional medicine to treat inflammation, herpes, kidney diseases, diarrhea, antidote against animal poisoning, urethra inflammations, fungus skin diseases and stomachaches. Leaf extract of the plant has anti-plasmodial activity, anti-HIV, hypoglycemic, analgesic, antimalarial, and antioxidant properties (Niyonzima, 1999).

MATERIALS AND METHODS

Collection of plant samples

Apparently healthy leaves of *S. campanulata w*ere collected from a forest in Akure, Ondo State of Nigeria. The plant leaf was identified by Prof Oyun M. B. of Forestry and Wood Technology Department, Federal University of Technology, Akure, Ondo State, Nigeria, and the voucher specimen was deposited in the University herbarium with number AF 1508.

Leaf extract preparations

At room temperature of 25±2 °C, leaves of *S. campanulata* were air-dried for seven days. The dried plant leaves were ground to smooth powder with a grinder (Thomas Wiley machine, model 5, USA). Two hundred grams (200 g) of the powder was extracted with 500 ml of ethanol at room temperature. The extract was concentrated in vacuo and stored in a refrigerator for use.

Acute toxicity test

With the criteria of WHO guideline for evaluation of efficacy and safety of herbal medicine (WHO, 2000) and Organization of Economic Co-operation and Development (OECD) guideline for testing chemicals (OECD, 2010), the extracts were tested *in vivo* for toxicity. Thirty-five mice of both sexes were purchased and quarantined for a week. Before the test, the mice were fasted for six h and divided into seven groups of five. Group 1 mice were each orally dosed with 10 ml/kg body weight of normal saline, while the mice in groups 2 – 7 were each dosed with 200, 500, 1000, 2000, 3000, and 4000 mg/kg body weight of extract, respectively. Toxic symptoms, according to the criteria of Lorke, (1983) were observed on the mice for 28 days, and Lethal $Dose_{50}$ (LD_{50}) of the quote was estimated by using the method of Miller and Tainter (1994). The LD cut-off of the quote was at 3000 mg/kg

body weight, and the therapeutic extract dose for this study was between 200 to 2000 mg/kg body weight. The performed experimental procedures on the animals were approved by Nigerian National Health Research Ethics Committee with the assigned number NHREC/08/2016.

Test bacteria species

Clinical *Salmonella typhi* was obtained from the research laboratory of the Microbiology Department, Federal University of Technology, Akure. The bacteria species was purified and cultured on Salmonella-Shigella Agar for confirmation of essential cultural characteristics. The pure isolate was Gram-stained and identified with biochemical tests for verification before stored on agar slant for use.

Experimental animals

Sixty Swiss albino mice of between 4 to 5 months with the body weight of between 23 to 35 grams were used. The mice were acclimatized for two weeks by feeding them with regular rat feed and water. After which, they were denied food for 18 h but with access to water only and conducted in compliance with the NIH guide.

Experimental design

Twelve groups of five mice each were conducted for the experiment. Group, I was allowed access to feed and water as the negative control. Group II mice were dosed with 1 g/kg body weight of paracetamol (positive control) three times daily for three days. Groups III-VII were each infected orally in a single dose per day for three days with 1 ml of 10^3 CFU/mL of *Salmonella typhi* and treated with 200, 400, 800, 1000, and 2000 mg/kg body weight of extract concentrations respectively for three days. Groups VIII-XII were orally dosed with 1 g/kg body weight of paracetamol in a single dose for 3 days and treated with 200, 400, 800, 1000, and 2000 mg/kg body weight of extract concentrations respectively for three days. After the experimental procedures, mice in each group were anesthetized, and the jugular vein of each mouse was cut with head held downwards and allowed to bleed into a vacutainer blood collection tube. The blood collection tubes were labeled according to groups of mice. For liver function test and histopathology, the mice in each group were dissected, and liver tissues were collected for analysis.

Hematology of experimental mice

Red blood cells and white blood cells, leucocytes differential counts, neutrophil, monocyte, eosinophil, and lymphocyte differential counts were estimated by the criteria of Dacie and Lewis (2002) with the automated hematologic analyzer SYSMEX KX21 (SYSMEX Corporation, Japan), hemoglobin was evaluated with the use of Sahli's Hemoglobinometer by standard procedures according to the criteria of Wintrobe et al. (1961); D'Amour et al. (1967), albumin was by the technique of Doumas et al. (1971) total cholesterol by the technique of Abel et al. (1953), urea with the technique described by Fenech and Tommasini (1952), creatinine by the method of Lustgarten and Wenk, (1972) and total bilirubin by the process of Watson and Rogers (1961).

Histopathology of liver tissues

Mice from the controls, extract-treated, bacterial treated, and satellite groups, liver samples were collected and washed with normal saline. Small pieces of liver was cut from each treatment and dehydrated in grades of ethanol. Xylene was used to clear traces of ethanol and water from the tissues after dehydration before impregnating then in paraffin wax for 1 h at a controlled temperature of 60 °C. The tissues were after that embedded in molten paraffin wax and sectioned with a microtome (Bright, England) at 4 - 6 µm. The sectioned tissues were floated in a water bath regulated at 35 °C and picked with slides previously robbed with egg albumin. The tissues were then dewaxed with xylene, hydrated, cleared with xylene, stained with hematoxylin and eosin, and mounted with Dibutylphthalate Polystyrene Xylene (DPX). The prepared slides were then allowed to dry and photographed, which were then observed with a binocular microscope for the level of damages or safety.

Statistical analysis

Obtained results from this study were expressed as Mean±SD. Differences were compared by One-way Analysis of Variance (ANOVA) and were followed by Dunnett's Multiple Comparison Test using SPSS version 16.

RESULTS AND DISCUSSION

Effect of *S. campanulata* on hematology of mice

In this study, a mice model was used to study the hepatotoxic and hepatoprotective effects of *S. campanulata* ethanol leaf extract at different concentrations. Before the administration of the quotes to the paracetamol-induced mice and bacteria-infected, the LD_{50} of the plant extract was investigated and was found safe at 2800 mg/kg. Figure 1 represents the hematological profiles of positive control, negative control, bacterial infected, and paracetamol-induced (toxicant) mice and co-administered with the various extract concentrations (satellite). The red blood cells' mean count in the negative control was 7.6±70.92 million/mm^3, which was higher than the positive control with a value of 4.36±0.12 million/ mm^3. A lower WBC means count of 3.50±0.18 thousand/ mm^3 in negative control than the positive control group (9.62±0.39 thousand/ mm^3) was recorded. Also, a higher value above the permissible level (11-19%) was obtained in the hemoglobin of the positive control (20.11%) than the negative control (11.50%).

The plant extract concentrations of between 200 - 2000 mg/mL administered to groups of mice were to ascertain any physiological change that might result from the plant toxin. However, the results obtained suggest that *S. campanulata* ethanol leaf extract was not toxic to the mice at the evaluated concentrations. Also, the physiological changes exerted on the hematology profile of the mice due to bacterial infection, and paracetamol toxicity were improved by the extract's potency on dose dependent. Groups III-VII was proposed; hence drug is not administered without illness. The leaf extract of *S. campanulata* has been found to possess antimicrobial activity, and to ascertain its non-effect on organs for safe use in ailments, *S. typhi* infection was allowed to manifest illness in the mice. Outside it that physiological changes in mice were compared with the negative and positive control groups, the hematological, biochemical, and histopathological evaluations hold it safe for use; hence organs such as kidney and intestine were as well protected.

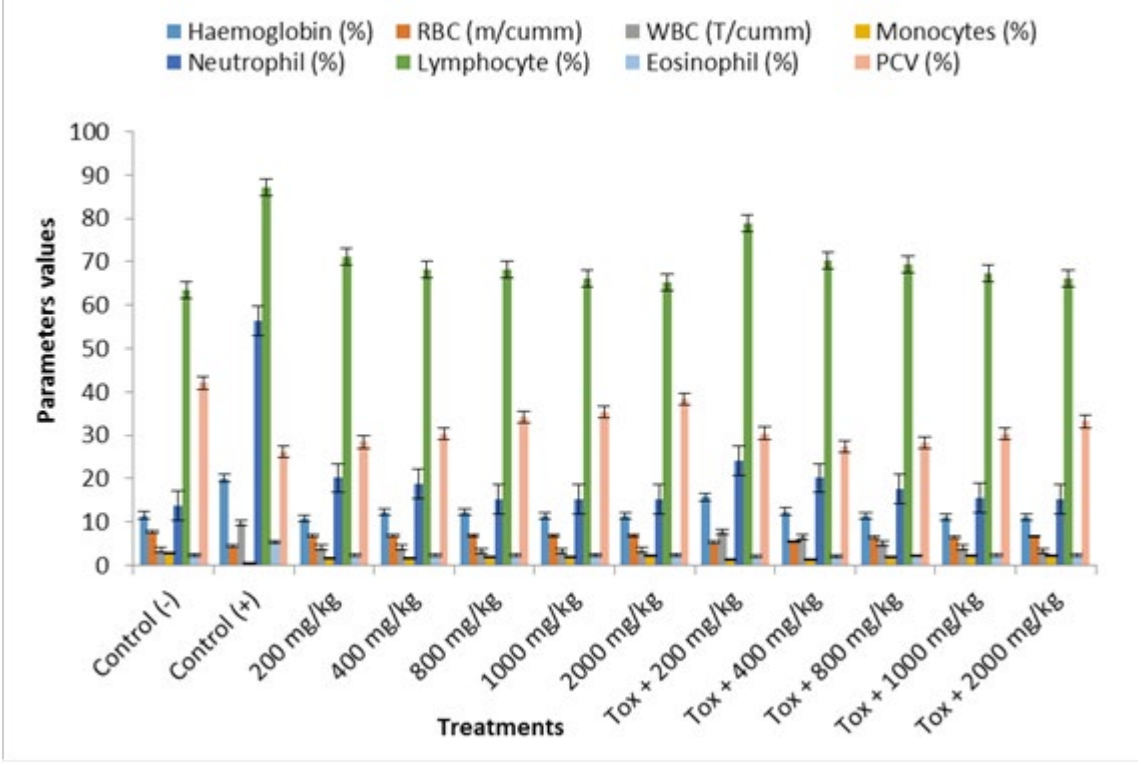

Figure 1. Effect of ethanol leaf extract of *S. campanulata* on hematology of albino mice

Effect of *S. campanulata* on biochemical in liver function

The liver is a multipurpose organ that helps to break down toxins in the body; therefore its damage by any hepatotoxic agent is of necessary consequence (Ansah, 2013). *S. campanulata* ethanol leaf extract effectiveness in biochemical markers to ascertain safety and toxicity of its therapeutic use is presented in Table 1. Total protein was 7.15±0.45 and 5.18±0.60 g/dL, respectively for, negative and positive controls. This significant decrease in the positive control mice suggests depletion of protein which is having the potential of increasing hepatic damage that can result in severe liver necrosis. However, the rise in total protein contents in the groups treated with 200-2000 mg/kg^{bw} of the extract was 6.34±0.40, 6.48±0.28, 6.53±0.28, 6.56±0.26, and 6.61±0.34g/dL respectively. This observed result suggests improvement in total protein alongside extract concentrations. Also observed in the satellite groups was the increase in protein towards normalcy alongside extract concentrations with values of 4.78±0.03, 5.22±0.14, 5.38±0.24, 5.42±0.06, and 5.61±0.30 g/dL in the toxicant treated and co-administered with 200-2000 mg/kg^{bw} of extract. Treatment with the plant extracts protected protein depletion initiated by paracetamol toxins in the mice and was confirmed by the observed gradual increase in protein level. This could be possible because of the essential phytochemicals present in the leaf extract. Bhadauria et al. (2007) have reported that the presence of various flavonoids and esters present in plants might be responsible for the stimulation of protein biosynthesis.

Urea level in the biochemical marker of the liver function of mice treated with the extracts was 18.15±1.50 mg/dL in the negative control and 23.40±0.16 mg/dL in the positive control. These results emphasized adverse impact on the liver with the induced paracetamol and positive effects on health status with regular rat meal and water. Recorded result in the treated mice with 200 - 2000 mg/kg^{bw} of the extract was in decreasing order of 22.65±0.42, 22.44±0.23, 21.60±0.28, 21.43±1.18, and 21.24±1.03 mg/dL, respectively. In the satellite mice groups induced with toxicant and co-administered with extracts, an increase in urea values alongside extract concentrations from 26.24±0.46 - 22.18±0.02 mg/dL was observed. This observation definitely illustrates urea reduction in circulation with the extract treatments. A decrease in values to normalcy, as suggested by the results obtained from the negative control, was also observed in the uric acid, creatinine, and cholesterol parameters. So from all observations of the biochemical parameters involved in the liver function of the mice with the leaf extract, indicates recovery of the injured liver from 200 mg/mL extract concentration. The wide difference in these values exists between the satellite groups of mice, where values of between 200 - 2000 mg/kg^{bw} of the extract are 59.56±1.68, 56.00±3.64, 56.06±1.16, 55.04±1.75, and 55.68±2.65 U/L, respectively. Despite this, the effectiveness of the quote on liver recovery from the sustained injuries imposed by paracetamol was evidenced in the decreased values alongside extract concentrations when compared with the positive control value. A similar trend of decrease in values to normal condition in the release of bilirubin into circulation to predict of non-toxicity of the extract even at 2000 mg/mL concentration was magnificent. Total albumin was found higher in the negative control (4.90±0.11 g/dL) than the positive control (2.21±0.60 g/dL).

While the results of bacteria/extract-treated mice ranged from 2.14±0.25 g/dL in the low dose of 200 mg/kg^{bw} of extract to 3.88±0.09 g/dL in the medium dose of 400 mg/kg^{bw} extract, it was 4.28±0.27 g/dL in the high amount of 800 mg/kg^{bw} of extract and 4.55±0.36 g/dL in the overdose of 2000 mg/kg^{bw} of extract. However, the decrease in value towards meeting up with the negative value with extract concentrations as observed in total protein, urea, and total albumin, was also the order in bilirubin, uric acid, creatinine, and cholesterol levels. Paracetamol administration at overdose to the mice initiated increase in cholesterol,

bilirubin, urea, uric, acid and creatinine levels. On the other hand, the lower values in protein and total albumin observed in the negative group are promising signs of liver function and integrity. The significant increase in the paracetamol-induced mice values in cholesterol, bilirubin, urea, uric acid, and creatinine levels indicated various damages such as hepatic, myocardial, and renal damage and; are responsible for skeletal muscle alteration (Mada, 2014). The ability of overdose with paracetamol to effect damages on liver tissues is connected with the observed varieties of alteration in hematological parameters; and indeed of sufficient injuries to manifest intracellular constituents into circulation. The amount of these in circulation as recorded is a prediction of hepatocellular damages. Stabilization activities of the hematological parameters investigated with the treatment of the plant extract concentrations manifested distinct improvement in the functional status of liver cells. They could be due to free radical scavenging action of the extract. Bilirubin, in its nature, is toxic and was recorded in circulation as a result of a breakdown of hemoglobin. This bilirubin in circulation, would have been carried to the liver for detoxification and excretion but was unable to bind to albumin as its function was also adversely affected by the toxicant. Gagliano et al. (2007), have reported that overdose of paracetamol elicits injury to hepatic parenchyma that will cause a high increase of bilirubin in circulation. The extracts of *S. campanulata* prevented the severity of liver damage caused by paracetamol, as evidenced by the low level of bilirubin in the serum. A similar result was reported by Panchal et al. (2013). Albumin level was shallow; hence the liver that produces it was defective. The low level of bilirubin in the extract-treated mice, further confirmed that the damages observed in the positive control mice were as a result of a high dosage of paracetamol. The results obtained in this study is following with Gowda et al. (2010), whose report stated that biochemical markers play an important role in accurate diagnosis and also for assessing risk and adopting therapy that improves clinical outcomes and also with Mohamed et al. (2010), who stated that popularity of herbal remedies is increasing globally and at least one-quarter of patients with liver disease use ethnobotanicals.

Table 1. Effect of ethanol leaf extract of *S. campanulata* on biochemical parameters in liver functions of albino mice.

Group	Bilirubin (mg/dL)	Total albumin (mg/dL)	Total protein (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)
Control (-)	1.08±0.10	4.90±0.11	7.15±0.45	18.15±1.50	6.05±0.60	1.38±0.30	113.70±0.50
Control (+)	2.11±0.63	2.21±0.60	5.18±0.60	23.40±0.16	8.02±1.10	1.96±0.09	218.14±0.64
200mg/kg^{bw}	1.65±0.10	2.14±0.25	6.34±0.41	22.65±0.42	8.36±0.14	1.78±0.44	156.03±6.05
400mg/kg^{bw}	1.56±0.34	3.88±0.34	6.48±0.28	22.44±0.23	8.22±0.63	1.75±0.18	151.42±1.15
800mg/kg^{bw}	1.35±0.18	4.28±0.27	6.53±0.28	21.60±0.28	7.30±1.07	1.62±0.65	142.13±0.47
1000mg/kg^{bw}	1.26±0.21	4.35±0.16	6.56±0.26	21.43±1.18	7.21±0.13	1.51±0.18	133.21±0.13
2000mg/kg^{bw}	1.12±0.03	4.55±0.36	6.61±0.34	21.24±1.03	6.18±0.45	1.47±0.28	130.16±0.18
TOX+200mg/kg^{bw}	1.45±0.26	2.84±0.26	4.78±0.03	26.24±0.45	8.40±0.23	2.40±0.14	222.43±0.56
TOX+400mg/kg^{bw}	1.37±0.44	2.88±0.34	5.22±0.14	26.18±0.21	8.26±0.26	2.28±1.03	163.07±1.13
TOX+800mg/kg^{bw}	1.28±0.31	3.72±0.18	5.38±0.26	25.31±0.17	7.48±1.17	1.66±0.24	157.14±0.84
TOX+1000mg/kg^{bw}	1.20±0.11	3.60±0.18	5.42±0.16	23.43±0.11	6.40±0.12	1.53±0.33	130.21±1.63
TOX.+2000g/kg^{bw}	1.15±0.40	3.65±0.09	5.61±0.30	22.18±0.02	6.33±0.27	1.50±0.26	125.08±1.13

Legend: Tox = Toxicant (paracetamol) co-treated with extract concentrations

Histopathology of experimental mice liver

The histopathological sections of the liver tissue as seen under the light microscope on the bacteria/extract-treated mice, the positive, negative controls, and the satellite-co-administered with extracts treatment are shown in Figures 2a and 2b.

Observed from the negative control mice are no distortion. The liver cells appeared normal in shape with a prominent nucleus, a precise central vein with typical architectural structure, and a well-preserved cytoplasm. Hepatic cells were arranged in cord-like fashion, which is well separated by sinusoids. The mice dosed with 1g/kg of paracetamol (positive group), has severe hepatocellular degeneration. The representative of liver mice infected with *S. typhi* and co-treated with 200 mg/kg body weight of extract showed no adverse effects in the liver except a hepatocellular necrosis. In the liver of mice induced with paracetamol and co-administered with 200 mg/kg body weight of extract concentrations, recovery from damages was observed as sinusoids, hepatocellular necrosis, portal vein, and dented hepatic sinusoid were seen. The kidney and intestine; sectioned tissues of mice infected with *S. typhi* and co-administered with the plant extract concentrations are presented in Figure 3.

Evidence of recovery from distortion of the organs were observed in the sectioned kidney and intestine; after treatment. However, the kidney and intestine; sectioned tissues of mice during bacterial infection had no distortions, but distortions were observed in the biochemical and hematology parameters evaluated. The administered concentrations of the plant extracts of between 200-2000 mg/kg, were found not toxic based on values obtained, which were not significantly different from the negative control values. However, they were also found to reduce the elevated importance of hematological parameters in the satellite mice groups towards normality. Activities of the plant extract concentrations in hepatoprotective could be dependent on the antioxidant values of free radical scavenging efficacy of the plant extract (Akharaiyi, 2015). Several plant chemicals have been shown to act synergistically as antioxidants. In this study with *S. campanulata* ethanol extract, it further strengthened the report that without reliable modern drug to manage liver protection, herbs can serve as an alternative to solve liver problems (Buraimoh, 2010; Arifianti, 2020).

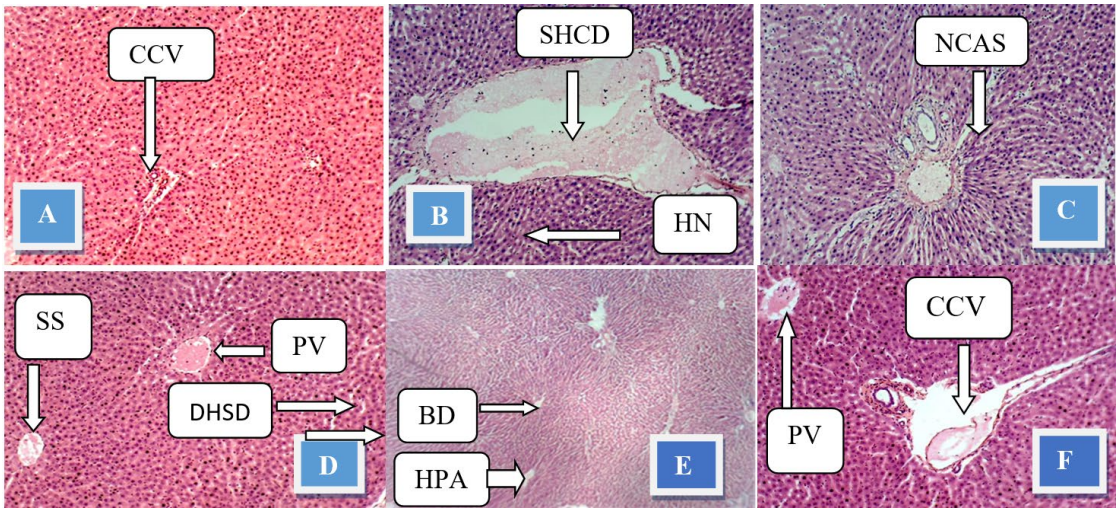

Figure 2a. Histopathological sections of the liver. (A – Mice fed with regular diet and water (-v control), (B - Mice dosed with 1 g/kg body weight of paracetamol without treatment (+v control), (C - Mice infected with bacteria and treated with 200 mg/kg body weight of leaf extracts), (D - Paracetamol induced and co-administered with 200 mg/kg body weight of extract), (E- Mice infected with bacteria and treated with 400 mg/kg body weight of leaf extracts), (F - Paracetamol induced and co-administered with 400 mg/kg body weight of extract).

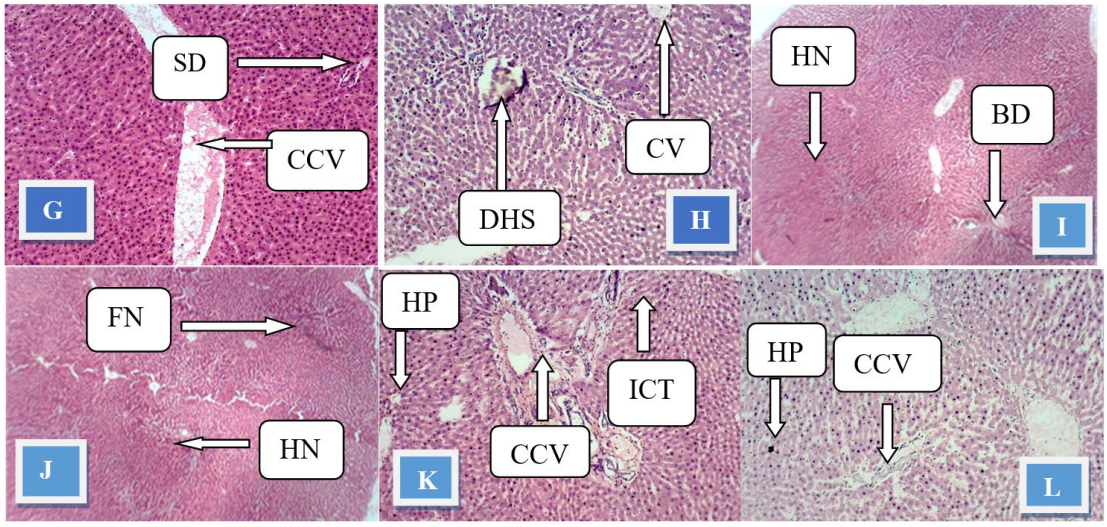

Figure 2b. Histopathological section of the liver. (G - Mice infected with bacteria and treated with 800 mg/kg body weight of leaf extracts), (H - Paracetamol induced and co-administered with 800 mg/kg body weight of extract), (I - Mice infected with bacteria and treated with 1000 mg/kg body weight of leaf extracts), (J - Paracetamol induced and co-administered with 1000 mg/kg body weight of extract), (K- Mice infected with bacteria and treated with 2000 mg/kg body weight of leaf extracts), (L - Paracetamol induced and co-administered with 2000 mg/kg body weight of extract).

Legend: Clear central vein (CCV), Hepatocellular necrosis (HN), Sinusoids (SS), dented hepatic sinusoid (DHSD), Portal vein (PV), Normal cellular architecture (NCAS), Severe Hepatocellular Degeneration (SHCD), Bile duct (BD), Interlobular connective tissue (ICT), Hepatocytes (HP), Central vein (CV), Dented hepatic sinusoid (DHS), Sinusoids (SD), Focal necrosis (FN) and Hepatic artery (HPA)

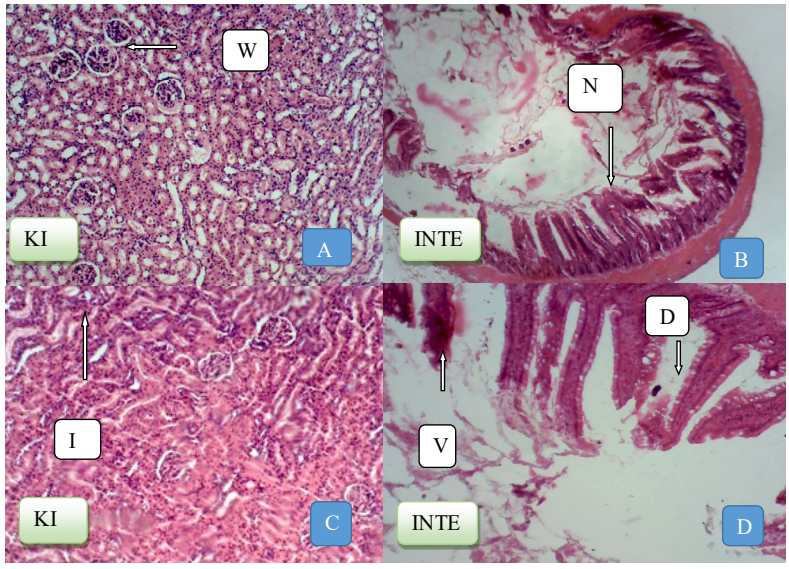

Figure 3. Histopathological sections of Kidney and intestine; of mice (A= Kidney of mice after treatment from *S. typhi* infection, B = Intestine of mice after treatment from *S. typhi* infection, C = Kidney of mice with *S. typhi* infection, D = Intestine of mice with *S. typhi* infection.

Legend: Well structure glomeruli (WSG), Normal villi architectural structure (NVAS), interstitial space (IS), Villi inflammation (VIF), distorted villi structure (DVS).

CONCLUSION

On inducing some mice with an overdose of paracetamol, specific negative changes for liver malfunction were observed. Also, changes from negativity to normality resulting from healing or injury recovery were noticed on a group of mice pretreated (satellite) with the plant extract at varying concentrations. The evaluation of these comparisons was possible because of the negative control group of mice which were given regular mice feed and water only. Most of the pathological changes observed in the mice liver include fibrosis around the central vein and sinusoids which were reduced by the plant extract concentrations in the satellite group of mice. The hepatoprotective effects of *S. campanulata* ethanol leaf extracts highlight their unquestionable antioxidant properties. In the hematological assay, higher values in positive control than negative control was observed in bilirubin and total protein. The rise in these parameters signifies less functional activity of the liver. Apart from the increase in bilirubin and total protein, specific adverse effects were also observed in the urea, uric acid, creatinine, and cholesterol. These alterations indicated skeletal muscle. The hepatoprotective effects of the extract of *S. campanulata* against paracetamol-induced toxicity in this study were further confirmed by the histopathological studies.

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CONFLICT OF INTEREST

There is no conflict of interest among authors of this manuscript.

AUTHOR CONTRIBUTION STATEMENT

Developing the hypothesis, experimentation, first draft of the text, analysis, and interpretation of results (Akharaiyi, F. C.), literature search, statistics, and assistant in the research experimentation (Okafor, A. C.), reviewing the text and approval of the final manuscript before sending out for publication (Akharaiyi, F. C., Okafor, A. C.).

REFERENCES

Abel L. L, Levy B. B., Brodie B. B., Kendall, F. E. (1953). A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *Journal of Biology and Chem*istry, *195*(1), 357–66.

Pianaro, A., Pinto, J. P., Ferreira, D. T., Ishikawa, N. K., Braz-Filho, R. (2007). Iridoidglucoside and antifungal phenoilic compounds from *Spathodea campanulata* roots. Iridoidglucoside and antifungal phenoilic compounds from *Spathodea campanulata* roots. *Ciências Agrárias, Londrin*, *28*(2), 251-256.

Akharaiyi F. C, Boboye B. E., Akpambang V. O., Adetuyi F. C. (2015). Phytochemical and antioxidant effect of *Spathodea campanulata* leaf extracts. *International Journal of Biochemistry Research and Review*, *7*(3), 148-159. doi: 10.9734/IJBcRR/2015/16371

Ansah C, Dadzeasah P. E., Asiamah E. (2013). Aqueous stem bark extract of *Spathodea campanulata* (P. Beauv) modulates carbon tetrachloride induced hepatic damage in rats. *American Journal of Pharmacology and Toxicology*, *8*(1), 39-50 doi: 10.3844/ajptsp.2013.39.50

Arbab A. H, Parvez M. K, Dosari M. S, Rehaily A. J, Ibrahim K. E, Alam P. (2016). Therapeutic efficacy of ethanolic extract of *Aerva javanica* aerial parts in the amelioration of CCl4- induced hepatotoxicity and oxidative damage in rats. *Food Nutrition Research*. 60:30864. https://doi.org/10.3402/fnr.v60.30864

Arifianti, Lusiana., Sukardiman, Sukardiman., Indriyanti, Niken., Widyowati, Retno. (2020). Anticancer property of *orthosiphon stamineus* benth. Extracts in different solvent systems against t47d human breast cancer cell lines. *FABAD Journal of Pharmaceutical Sciences*, *45*, 3, 187-194

Bhadauria, M., Satendra, N., Sanyam, S. (2007). Hepatoprotective efficacy of propolis extract: A biochemical and histopathological approach. *Irani Journal of Parmacology and Therapeutics*, *6*(2), 145-154.

Buraimoh, A. A. Bako, I. G., Ibrahim, F. B. (2010). Hepatoprotective effect of ethanolic leaves extract of *Moringa oleifera* on the histology of paracetamol induced liver damage in Wistar rats. International journal of *Animal and Veterinary Advances*, *3*(1), 10-13

Dacie, J. V., Lewis, S.M. (2002). Practical Haematology. 11th Edn., Elsevier, London, UK; pp: 380-382.

D'Armour, F. E., Blood, F. R., Belden, D. A. (1965). The Manual for Laboratory Work in Mammalian Physiology. 3rd ed. Illinois Chicago, The University of Chicago Press, 4-6.

Deshwal N, Sharma A. K., Sharma P. (2011). Review on hepatoprotective plants. *International Journal of Pharmaceutical Sciences Review and Research*, *7*, 15-26.

Doumas, B. T., Watson, W. A., Biggs H. G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimaca Acta, 31*(1), 87–96. doi: 10.1016/s0009-8981(96)06447-9

Fenech, G., Tommasini A. (1952). Method of colorimetric determination of urea. *Bollettino Chimico Farmaceutico*, *91*(10), 391–395.

Gagliano, N., Grizzi, F., Annoni G. (2007). Mechanism of aging and liver functions. *Digest Discovery Science*, 25, 118-123. doi: 10.1159/000099475

Gowda, S., Desai, P. B., Kulkarni, S. S., Hull, V. V., Math, A. A. K., Vernekar, S. N. (2010). Markers of renal function tests. North American Journal of Medical Science, *2*(4), 170-173.

Lorke, D. A (1983). new approach to acute toxicity testing. *Architect Toxicology*. *54*, 275-287. doi: 10.1007/bf01234480

Lustgarten, J. A, Wenk R. E. (1972). Simple, rapid, kinetic method for serum creatinine measurement. *Clinical Chemistry*, *18*(11), 1419–1422.

Khan, M. Z., Jogeza, N., Tareen, J. K., M., Imran Shabbir. M., Malik, M. A., Khan, A. R. (2016). Compilation on medicinal plants with hepatoprotective activity. *SRA Medical Journal*, *8*(3), 196-202

Mada, S. B., Inuwa, H. M., Abarshi, M. M., Mohammed, H. A., Aliyu, A. (2014). Hepatoprotective effect of *Momordica charantia* extract against CCl4 induced liver damage in rats. *British Journal of Pharmaceutical Research*, *4*(3):368-380. doi: 10.9734/BJPR/2014/4885

Mohamed, A. A., Khalil, A. A., El-Beltagi, H. E. S. (2010). Antioxidant and antimicrobial properties of kaff maryam (Anastatica hierochuntica) and doum palm (Hyphaene thebaica). *Grasas Y Aceites*. *61*(1):67–75. doi: 10.3989/gya.064509

Miller, L. C., Tainter M. C. (1994). Estimation of LD50 and its error by means of logarithmic-probit graph paper. *Proceeding of the Society of Experiment Biology and Medicine, 57*, 261-264.

Ngouela, S., Tsamo, E., Sondengam, B. L., Connolly, J. D. (2001). Spathodol a new polyhydroxysterol from the leaves of *Spathodea campanulata*. *Journal of Natural Products, 54*(3), 873-876.

Niyonzima, G., Laekeman, G., Witvrouw, M., Van Poel, B., Pieters, L., Paper, D., De Clercq, E., Franz, G., Vietinck, A. J. (1999). Hypoglycemic, anticomplement and anti-HIV activities *Spathodea campanulata* stem bark. *Phytomedicine*, *6*(1), 45-49. doi: 10.1016/S0944-7113(99)80034-8

Panchal, C. V., Jyotiram, A., Sawale, B., Poul, N. Khandelwal, K. R. (2013). Hepatoprotective activity of *Lageneria sicerarla* (MOLINA) Standley fruits against paracetamol induced hapatotoxicity in mice. *International Journal of Pharmaceutical Science Research*, *4*(1), 371-377. doi: 10.13040/IJPSR.0975-8232.4(1).371-77

The Organization of Economic Cooperation and Development (OECD). (2010). The OECD guideline for testing of chemical: 420 Acute Oral Toxicity. France.

Watson, D., Rogers, J. A. (1961). A study of six representative methods of plasma bilirubin analysis. *Journal of Clinical Pathology*, *14*, 271–278.

Wintrobe, M. M., Lee, G. R., Boggs, D. R., Bithel, T. C., Athens, J. W., Foerester J. (1961). Clinical Hematology, 5th ed. Philadelphia, Les and Febiger, p.326.

World Health Organization (2000). General guidelines for methodologies on research and evaluation of traditional medicine. Switzerland.