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Evaluation of Histomorphological, Toxicological and Antimicrobial Activities of Ethanolic Extract of *Calliandra portoricensis* Root in Rodents

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Authors' contributions

This work was carried out in collaboration between all authors. Author VNE designed the experiment and conducted the microbial study. Author SOO partook in the experimental design and in the write up of the work. Author GOM undertook the tissue processing and analysis as well as partook in the write up and final editing of the manuscript. Author JEE partook in the editing of the manuscript and also performed the statistical analysis. Author DAO conducted the biochemical analysis. Author PO carried out the laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To evaluate histomorphological, toxicological and antimicrobial activities of ethanolic extract of *Calliandra portoricensis* root in rodents.
Introduction: *C. portoricensis* is usually administered for a lengthy period in treating diseases like

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dysmenorrheal, rheumatism and convulsion.
Methods: Microbial purity was evaluated on some bacterial and fungal organisms. Toxicity of the extract was evaluated in Swiss albino mice by administering graded oral doses of the extract from 1.0 to 20.0 g/kg body weight (bwt). Wistar rats were fed different doses of the extract for 30 days to evaluate their biochemical profiles while vital organs were processed for histology.
Results: Extract inhibited *Enterococcus faecalis* and *Streptococcus pneumonia* ATCC 49619 organisms at 150, 300 and 600 mg/ml with the inhibitory diameters of 13.0, 14.0 and 16.0 mm for *E. faecalis* and 10.0, 11.0 and 13.0 mm for *S. pneumonia*. Median acute toxicity (LD_{50}) was 5.0 g/kg bwt. Significant increase ($p \leq 0.05$) in aspartate aminotransferase and alanine aminotransferase occurred while hepatic tissue morphology showed sinusoidal and portal congestions. Also significant increase ($p \leq 0.05$) in the plasma creatinine and urea occurred.
Conclusion: *C. portoricensis* showed to be an effective antibacterial agent and exhibited no toxic effect at normal dose. However administration above the recommended dose might be injurious to the liver.

Keywords: Calliandral portoricensis; anti-microbial; toxicological; histo-morphological; rodents.

1. INTRODUCTION

Plants from the outset have served as veritable sources of food to sustain human and animal lives in addition to their other economic benefits. The discovery that some plants derived chemical constituents could serve as useful and important therapeutic weapons against human and animal diseases has made plants *a sine qua non* to human and animal lives. The increasing popularity in the use of herbal remedies to treat diseases of various origins could be attributed to their advantages of being a cheap source of medical care [1]. Besides, there is a growing disillusionment with modern medicine coupled with misconception that herbal remedy being natural may be devoid of adverse and toxic effects associated with allopathic medicines. Owing to this misconception, herbal drugs are administered in most disease conditions over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage [1]. The danger associated with the potential toxicity of such therapies used over a long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs [2,3].

There is also a serious concern that the preparation of herbal medicine by the traditional healers are in most cases conducted in an unhygienic condition resulting to contamination with microbiological and foreign materials such as heavy metals, pesticide residues or even aflatoxins [4]. Contaminants when present in an herbal preparation may lead to serious health defects underscoring the claimed safety. An increase in the morbidity and mortality associated with the use of herbal or the so called traditional medicines has raised universal attention in the last few years [5]. Upon exposure, the clinical toxicity may vary from mild to severe and even life threatening making the safety and toxicity evaluations of these preparations imperative.

Calliandra portoricensis (C. *portoricensis*) is a member of Fabaceae family. It is a shrub or little tree which is 6 meters tall with evergreen small bipinnate leaves, whitish- pink scented and globose flowers. It is native to Central America like Mexico, Panama and West Indies with some species also found in West Africa particularly in Nigeria [6,7]. The plant is noted for its anti-microbial activities and is also used by herbalists in Nigeria for other health benefits. Its root is employed in the preparation of various herbal medicines often administered for a very long period in the treatment of disease conditions such as dysmenorrheal, rheumatism and convulsion [8] making the use of this herbal preparation very popular among women. The prolonged use of this medicinal plant for medication suggests the likelihood of an abuse thereby undermining the potential histo-toxicity effects that might occur in the vital organs.

Considering that the root of *C. portoricensis* usually have prolonged period of administration in the treatment of diseases there is however scanty information on the effect of the plant root on histomorphology of the vital organs as well as its antimicrobial profile. This study therefore was aimed at evaluating the histo-toxicity and antimicrobial activities of ethanol root extract of *C. portoricensis*.

2. MATERIALS AND METHODS

The plant *C. portoricensis* was identified by a taxonomist with vouchers no LUH 4598. The roots were collected, washed with copious amount of water to remove sands and water then allowed to dry. They were cut into small pieces and dried in an oven at 40°C and were coarsely powdered. A total of 850 g weight of the coarse powder was extracted with 5 L of 40% ethanol by maceration with frequent stirring for five days. It was filtered with no 4 Whitman paper, initially concentrated in vacuums in a rotary evaporator and finally lyophilized to yield 52.62 g dry powder (6 %).

2.1 Animals

Swiss albino mice (22.5 ± 2.5 g) of either sex were used for the acute toxicity study, while adult Wistar rats (130±15 g) were used for the sub-acute toxicity study. The animals were obtained from the Animal House of the College of Medicine of the University of Lagos. They were randomly selected with no preference for sex and were fed with a standard animal diet (Pfizer Feeds Ltd, Nigeria) and had access to water *ad libitum*. The studies were in compliance with the Institute of Laboratory Animal Research (ILAR) guidelines on the use and care of animals, in experimental studies [9]. They were maintained in spacious polypropylene cages in well ventilated animal house with 12 hrs dark and light cycle and were acclimatized for a week before the commencement of the study.

2.2 Test Bacteria and Growth Media

Gram positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis, Streptococcus pneumonia* ATCC 49619) and Gram negative (*Pseudomonas* species ATCC 27853, *Klebsiella species*, and *Escherichia coli* ATCC 259218) bacteria pathogens provided by Medical Microbiology laboratory, Nigeria Institute of Medical Research (NIMR), Yaba, Nigeria and Lagos University Teaching Hospital (LUTH), Lagos, Nigeria were chosen based on their clinical and pharmacological importance [10]. The stock cultures of these selected bacteria were grown on nutrient broth and then sub-culturedon MacConkey agar (oxide) at 37°C for 24 hrs except *Enterococcus faecalis (E. faecalis) and Streptococcus pneumonia* (*Strep. pneumonia)* ATCC 49619 that were sub-cultured on sheep blood agar (oxide) at 37°C for 24 hrs. The isolates were re-identified using standard microbiological methods and then maintained on Mueller-Hinton agar (MHA, Oxoid) slant at 4°C prior used.

2.3 Antimicrobial Activity

2.3.1 Determination of zone of inhibition method

In vitro antimicrobial activities of ethanolic root extracts of *C. portoricensis* against selected pathogenic bacteria were investigated by agar well diffusion method as described [11]. Overnight broth cultures of the respective bacteria strains were adjusted to turbidity equivalent to 0.5 McFarland standards, to yield approximately 2.0 x 10^6 colony forming unit (cfu)/ml.

The dried root extract of *C. portoricensis* was reconstituted with 5% ethanol to obtain a stock solution of 600 mg/ml from which 300 mg/ml and 150 mg/ml concentration were subsequently prepared. Wells of 8 mm were made into previously seeded TSA plates with 0.2 ml of 2 x 10^6 CFU of each test bacterium. Each well was filled with 0.2 ml of each concentration of the plant extract and antibiotic disc of Cefuroxime (30 µl) and Ofloxacin (30 µl) were used for positive control of Gram positive and Gram negative bacteria isolates, while solvent media was used as negative control respectively. The experiments were carried out under sterile condition and in duplicate for all isolates; plates were incubated for 24 hrs at 37°C. After incubation, zones of inhibitions were measured in millimeters (Table 1).

2.3.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC was determined using the method as described [12]. The working concentrations of the plant extract were mixed with the molten TSA that was allowed to cool for 45°C and then poured in Petri dishes. After solidification, 0.02 ml of 2 x 10^6 CFU/ml of test bacterial suspension was spotted at different points on the agar. The drops were allowed to diffuse for 30 minutes and the plates were incubated at 37°C for 72 hrs. The lowest concentration that did not permit any visible growth when compared with the control was considered as the MIC (Table 2).

The MBC was determined by sub-culturing the plates with no visible bacterial growth from MIC into TSA plate containing 3% Tween 80. The plates were incubated at 37°C for 24 hrs. The minimum bactericidal concentration was considered as the lowest concentration that could not produce a single bacterial colony [13] (Table 3).

2.4 Acute Toxicity Study

The toxicity study was carried out using thirty-five (35) male and female Swiss albino mice. The animals were randomly distributed into one control group and six treated groups containing five animals per group. They were maintained on animal cubes (Pfizer Feeds Nigeria Ltd), provided with water *ad libitum* and were allowed to acclimatize for seven days to the laboratory conditions before the experiment. After the overnight fasting, the control group received 0.3 ml 3% Tween 80. The doses, 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg bwt were respectively administered orally to the groups from Tween 80 solution of the formulation gel. The stock solution was prepared by dispersing 16 g of the gel with 7 ml of the Tween 80 solution in a 100 ml beaker and then transferred to a 20 mL volumetric flask. The volume was made to mark with the Tween 80 solution to give a stock solution of 800 mg/mL (80% w/v). For mice of average weight of 22.5 g was administered 20,000 mg/kg bwt (20 mg/g), the total volume consumed was 0.56 mL (450÷800 mL) while for 15,000 mg/kg bwt (15 mg/g) the total volume received was 0.42 mL. The animals were observed continuously for the first 4 hrs and then for each hour for the next 24 hrs and at 6 hourly interval for the next 48 hrs after administering the extract to observe any death or changes in general behaviour and other physiological activities [14].

2.5 Determination of LD_{50}

The median lethal dose (LD_{50}) was estimated for each group by log dose – probit analysis .The LD_{50} was calculated as the geometrical mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality [15].

2.6 Sub-acute Toxicity Study

Male and female Wistar rats were allowed to acclimatize to the laboratory conditions for seven days before use. The animals were maintained on standard animal feeds (Pfizer Nigeria ltd) and provided with water *ad libitum*. The animals were weighed and divided into four groups of five animals each and after the overnight fast of the animals the control group received a dose of 0.6 ml of 3% Tween 80 orally once a day for 30 days. The three treated groups respectively received the following doses: 100 mg/kg, 250 mg/kg and 500 mg/kg bwt of the gel orally once a day for 30 days [16-18]. The gel suspension (12%w/v) was prepared by dispersing the gel (12 g) with 45 ml of 3% Tween 80 solution in a beaker, and transferred to a 100 mL volumetric flask. Then the beaker was rinsed with the solution and the content transferred to the volumetric flask and volume made to mark with the Tween 80 solution.

The animals were weighed every seven days from the start of the treatment to note any weight variation. At the end of the experiment, the animals were starved overnight and on the 31st day, they were made unconscious by cervical dislodgement. The blood was collected via cardiac puncture in three tubes, one with EDTA for analysis of hematological parameters and the blood chemistry, fluoride oxalate tube for glucose analysis and with heparin to separate plasma for biochemical profiles. The heparinized blood was centrifuged within 5 min of collection at 4000 rpm for 10 min to obtain plasma which was analyzed for total cholesterol, total triglyceride, and HDL-cholesterol levels by modified enzymatic procedures from Sigma Diagnostics [19]. LDL-cholesterol levels were calculated using Friedwald equation [20]. Plasma was analyzed for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine by standard enzymatic assay methods [21]. Plasma glucose contents and protein contents were determined using enzymatic spectroscopic methods [22].

The blood samples were analyzed for red blood cells (RBC) by haemocytometic method [23], the haemoglobin (Hb) content was by Cyanmethaemoglobin (Drabkin) method [23] and packed cell volume (PCV) was estimated using the method as described by Ekaidem et al. [23]. Haematocrit tubes were filled with whole blood to the mark by capillary action and the bottom of the tubes sealed with plasticide and centrifuged for 4-5 minutes using haematocrit centrifuge. The percentage cell volume was read by sliding the tube along a “critocap” chart until the meniscus of the plasma intersected the 100% line. The white blood cell (WBC) count was as described by Ekaidem et al. [23].

2.7 Histology Slide Preparation

The animals were later sacrificed and the target organs, liver, kidney, heart, and testes harvested from each group and then fixed in 10% formal saline for seven days before embedding in paraffin wax. The fixed target organs were removed and dehydrated in increasing concentrations of alcohol; 70%, 80%, 90% and absolute alcohol (100%). The organs were treated with acetone and then cleared in xylene for 30 min to enhance the tissue transparency followed by impregnation and embedment in the paraffin wax. Each tissue was then sectioned at 5µm and cleared (dewaxed) for staining with haematoxylin and eosin (H &E) [24].

2.8 Statistical Analysis

Significant differences were determined using a Student's t-test. Differences were considered significant if p <0.05. All data were expressed as mean ± standard error of the mean.

3. RESULTS

3.1 Microbial Purity Study

This study revealed that the ethanolic extract of the root of *C. portoricensis* (Table 1) exhibited antibacterial activity against *E. faecalis* and *Strep. pneumonia* (ATCC 49619). At the concentrations of 150, 300 and 600 mg/ml, the inhibitory diameters of 13.0, 14.0 and 16.0 mm were observed against *E. faecalis* 10.0, 11.0 and 13.0 mm were observed against *Streptococcus pneumonia*. The extract exhibit no antibacterial activity against other bacterial strains tested at all the concentrations of the plant evaluated.

The MIC values (Table 2a) of the extract was 6.4 mg/ml for *Streptococcus pneumonia* (ATCC 49619), and for *Streptococcus facaelis*, the MIC value was 51.2 mg/ml.

The extract exhibited low MBC (Table 2b) value which was the same for both organisms.

3.2 Variation of Weights

The percentage difference in the weight of the treated animals compared to the control is shown in Fig. 1. Generally, there was insignificant ($p \geq 0.05$) decrease in the bwt of the treated animals compared to the control. A drop in bwt was observed in all the treated animals throughout the period under observation. The testicular weight however exhibited more significant decrease in the highest dose of the extract treatment. Macroscopic examinations showed no changes in the colour of the organs of the treated animals compared to the control.

Table 1. Inhibitory concentration of *C. portoricensis* extract at different concentrations on some bacterial organisms

Test bacteria pathogen	600	300	150
Enterococcus faecalis	16.0 mm	14.0 mm	13.0 mm
Streptococcus pneumonia ATCC 49619	13.0 mm	11.0 mm	10.0 mm
Staphylococcus aureus	NZ	NZ	NZ
Klebsiella species	NZ	NZ	NZ
Escherichia coli	NZ	NZ	NZ
Pseudomonas species	NZ	NZ	NZ

Hydro-ethanolic (40%) Plant Extract (mg/ml). Nz- no zone of inhibition

Table 2a. Minimum inhibitory concentration of the plant extract

Concentration of plant extract (mg/ml)	0.1	0.2	0.4	0.8	1.6	3.2	6.4	12.8	25.6	51.2
Streptococcus pneumonia ATCC 49619	+	+	+	+	+	+	-	-	-	-
Enterococccus faecalis	+	+	+	+	+	+	+	+	+	-

Table 2b. Minimum bactericidal concentration of the extract

Parameter	G (6.4 mg/ml)	J (51.2 mg/ml)
Streptococcus pneumonia	—	—
Enterococccus faecalis	+	—

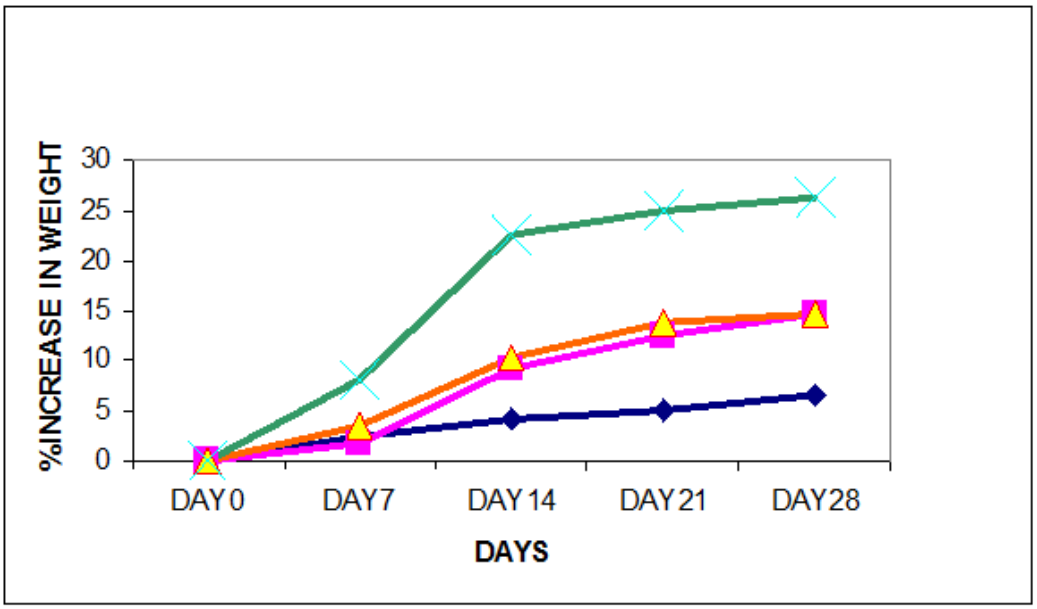


Fig. 1. Weight variation of animals treated with various doses of *C. portoricensis* and the control

♦ GPI (500mg/kgbwt) ■GPII (250mg/kgbwt) ▲GPIII (100 mg/kgbwt) X Control

3.3 Toxicological Studies

The acute toxicity study (Table 3) of *C. portoricensis* root extract showed no changes in the behaviour or effects in the sensory nervous system responses. All the animals that received 20.0 g/kg body weight dose of the extract died within 5 minutes of treatment while after 24 hours, 20%, 40%, 60% and 80% death were recorded respectively for the groups that received 2.5, 5.0, 10 and 15 g/kg bwt of the lyophilized extract. The LD_{50} value of the extract was calculated to be 5.0 g/kg bwt. The effects of the extract on the body weight variation of the control and treated animals were summarized in Fig. 1. There were significant ($p \leq 0.05$) decreases in the body weight of the treated animals which were observed to be dose dependent. There were however, insignificant changes in the organ weights (Table 4) of the animals compared to the control except for testes where marked decrease occurred at higher doses of the extract treatment.

The effects of the extract on the biochemical parameters are summarized in Table 5. There were significant ($p \leq 0.05$) increases in the AST values at all doses in the treated animals compare to the control. Significant increase ($p \leq 0.05$) in ALT values was observed only in the groups treated with 500 and 100 mg/kg bwt. There was also a significant increase ($p \leq 0.05$) in the plasma creatinine and urea levels for all the groups treated with the extract. However, the bilirubin level showed dose dependent decrease. On the other hand, there was a significant increase ($p \leq 0.05$) in total protein level. The LDL-cholesterol and total cholesterol decreased in levels while the levels of triglycerides and HDL-cholesterol (250 mg/kg dose) exhibited an increase.

Table 3. Acute toxicity study of the ethanolic extract of *C. portoricensis*

Dosages of drugs (g/kg)	Number of animals	Number of dead animals	% cumulative death of mice
0.5	5	0	00
1.0	5	0	00
2.5	5	5	20
5.0	5	5	40
10.0	5	5	60
15.0	5	5	80
20.0	5	5	100

Control animals received 0.4ml each of 3 % Tween 80

Table 4. Weight variations of organs per 100 g body weight of the control and rats treated with ethanolic extract of *C. portoricensis* in the sub-acute study

Parameter	Liver	Kidney	Heart	Brain	Testes
Control	5.62±0.07	0.93±0.01	0.57±0.01	1.34±0.01	1.94±0.01
100 mg/kg	5.65±0.03	0.91±0.01	0.49±0.01	1.32±0.01	1.95+0.02
250 mg/kg	5.60±0.09	0.91±0.02	0.50±0.00	1.35±0.01	1.05±0.01**
500 mg/kg	5.60±0.03	0.84±0.01	0.51±0.00	1.26±0.01	1.51±0.02**

*N= 5, *p≤ 0.05,**p ≤ 0.01.Control animals were administered with 0.4ml of 3 % Tween 80 solution*

Table 5. The effects of the ethanolic extract of *C. portoricensis* on the biochemical parameters of the control and the treated rats in the sub-acute toxicity study

Biochemical profile	Group I	Group II	Group III	Group IV
AST i.u./L	503.20 ±1.3*	269.30±2.8*	407.80 ±1.3*	128.60 ±1.8
ALT i.u/L	48.20 ±0.8*	36.90 ±0.9	50.60 ±0.3*	38.90 ±0.5
T-BIL µmol/L	3.20±0.1**	3.60 ±0.2	3.80 ±0.03	4.30 ±0.1
CREAT µmol/L	45.35 ±1.0*	44.28 ±1.0*	37.39 ±0.7*	24.75 ±1.4
UREA mmol/L	8.40±0.1**	7.20 ±0.1	8.30 ±0.1**	6.40 ±0.2
GLU mmol/L	3.40 ±0.2	2.40 ±0.1	4.70 ±0.1**	3.10 ±0.11
ALB g/L	40.60±1.5**	36.50 ±2.0	40.20 ±2.2**	33.40 ±1.2
T.P g/L	74.70 ±1.2*	80.90 ±1.6*	71.70 ±1.0*	66.10 ±1.2
HDL mmol/L	0.20±0.0	0.30 ±0.0	0.20 ±0.02	0.20 ±0.0
LDL mmol/L	0.50 ±0.1	0.50 ±0.0	0.40 ±0.0**	0.60 ±0.0
CHOL mmol/L	1.96 ±0.1**	1.85 ±0.1**	1.80 ±0.1**	2.63 ±0.1
T.G mmol/L	1.06 ±0.1**	0.76 ±0.1	1.04 ±0.0**	0.58 ±0.1
ALP µ/L	124.40 ±2.5**	152.60 ± 2.4**	108.30 ± 1.2**	251.20 ±2.7

*Results were expressed as mean± sem. n=5, * p≤0.05, ** p≤0.01,*
Key: Group I (received 500 mg/kg bwt), Group II – (received 250 mg/kg bwt). Group III – (received 100 mg/kg), Group IV – (served as control but received 3 % Tween 80)

The effects of the lyophilized extract on the red blood cells (RBC) components and white blood cell differentials were summarized in Table 6. The RBC decreased in level at the highest dose treatment while exhibiting slight increase at the lower doses compared to the control. The Hb levels of the animals treated with the extract doses decreased respectively compared to the control. Insignificant increase (p≥ 0.01) in PCV value was observed in the rats treated with 100 and 250 mg/kg bwt doses of the extract respectively compared to the control whereas significant decrease (p≤ 0.01) occurred at the highest extract dose. The mean corpuscular volume (MCV) level increased at the lower doses whereas there was a decrease at the highest dose treatment. However, mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin MCH respectively exhibited insignificant (p≥ 0.01) decrease in levels except in 250 mg/kg treatment where the decrease in MCHC was marked.

Table 6. Haematological values of control and treatment rats with the ethanolic extract of *C. portoricensis* for 30 days in sub-acute study

Parameter	Group I	Group II	Group III	Group IV
RBC x 10^6	5.47 ± 0.2	6.23 ± 0.2	6.34 ± 0.3	6.22 ± 0.1
Hb(g/dl)	10.00 ± 0.1	11.50 ± 0.4	11.50 ± 0.1	12.20 ± 0.2
PCV (%)	34.70± 0.1**	46.60 ± 0.1	42.60 ± 0.1	40.50 ± 0.4
WBC x10^3	7.50 ± 0.4**	9.30 ± 0.2*	8.50 ± 0.4**	5.50 ± 0.2
MCV (FL)	63.50 ± 0.2	74.70 ± 0.3*	67.20 ± 0.2	65.10 ± 0.1
MCH (pg)	18.20 ± 0.2	18.40 ± 0.3	18.10 ± 0.1	19.60 ± 0.3
MCHC (g/dl)	28.70 ± 0.1	24.70 ± 0.1**	27.00 ± 0.1	30.10 ± 1.1

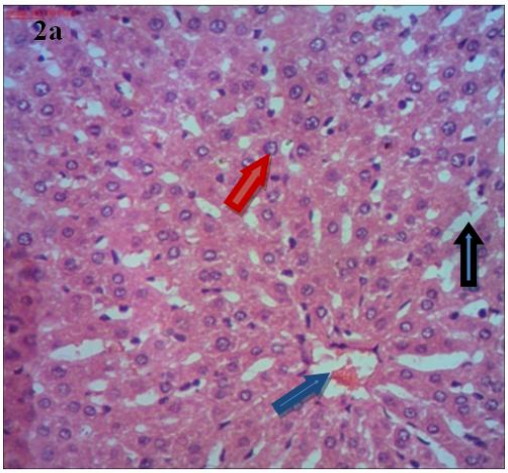
Key: Group I (received 500 mg/kg bwt), Group II – (received 250 mg/kgbwt). Group III – (received 100 mg/kg), Group IV – (served as control but received 3% Tween 80.)

3.4 Tissue Histo-morphology/Pathology

Figs. 2-5 showed the histological studies of the effects of the phytomedicine on target organs.

The hepatic tissue of the control (Fig, 2a) showed normal architecture. It exhibited typical parenchymal appearance though with indistinct hepatic lobules. The polygonal shaped hepatocytes were arranged as irregular cord-like structure interspaced by sinusoids which showed normal convergence towards the central vein. In the animals (Fig. 2b) treated with 500 mg/kg bwt of the extract, hepatic tissue showed severe hepatic sinusoidal and portal congestions and apparent edematous changes in the parenchyma. However, the hepatocytes showed no lesion.

The renal tissue of the control group (Fig. 3a) showed the renal corpuscles which appeared as rounded structure surrounded by narrow space, the Bowman's space. The cortical tubules seen in this section consisted mainly of proximal convoluted tubules with few of the distal convoluted tubules indicated. In the animals treated with 500 mg /kg bwt of the extract (Fig. 3b), no noticeable distortion were observed in the corpuscles and in the renal interstices respectively.



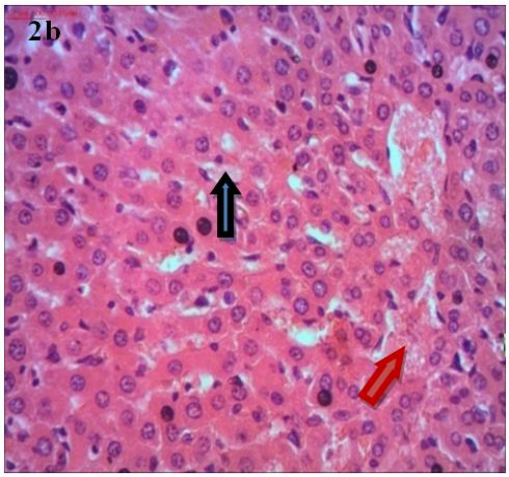
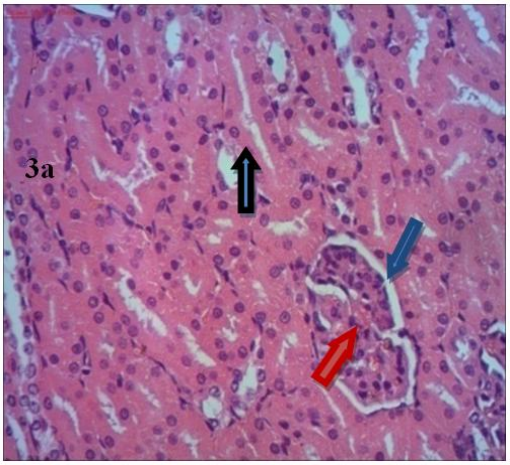


Fig. 2a. Photomicrograph (control) of a section of hepatic tissue showing hepatic sinusoid (black arrowed), central vein (blue arrowed) and hepatocyte (red arrowed)
Fig. 2b. The hepatic tissue of treatment with 500mg/kg bwt dose of the extract showed portal congestion (red arrowed) and sinusoidal congestion (black arrowed). Mag. X400



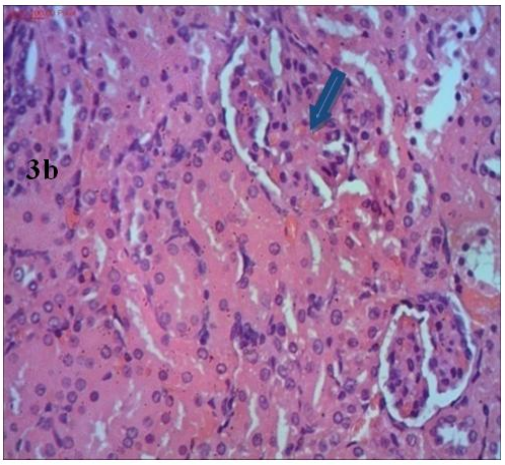


Fig. 3a. A section of cortical region (control) of renal tissue showing renal corpuscles (red arrowed), Bowman's capsule (blue arrowed) and convoluted tubule (black arrowed). Fig. 3b. The renal tissue of the treated animals at 500 mg/kg bwt dose showed no lesion. Mag. X400

Fig. 4a showed normal testis, the seminiferous tubules cut at different planes showed distinct boundary separated by interstitial spaces. The wall of the seminiferous tubules showed thick epithelium. Close to the basement of the thick epithelium is compactly arranged more primitive differentiating spermatogenic cell while the matured cells, spermatids and spermatocytes formed a cluster around the lumina. In the treated animals (Fig. 4b) there was less compact spermatogenic cell mass in the seminiferous tubules while no noticeable distortion was observed in the testicular interstice.

The cardiac tissue of the control group (Fig. 5a) indicating myocytes with deeply stained nuclei separated by unremarkable interstitium. In the treated animals (Fig. 5b), the myocytes showed no variation compared to the control.

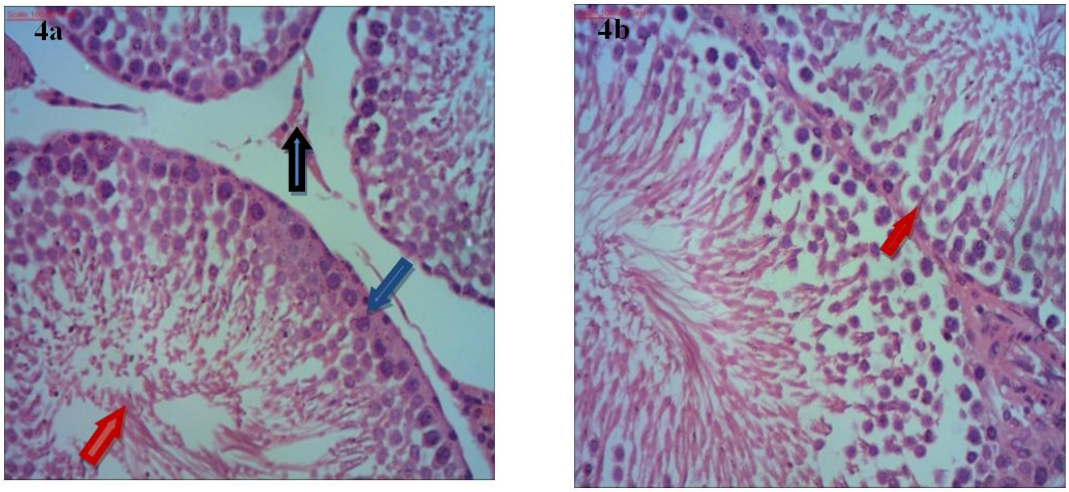


Fig. 4a. A section of testicular tissue (control) indicating densely packed spermatogenic cells (blue arrowed), interstitial cells of Leydig (black arrowed) and wavy tails of spermatozoa (red arrowed). Fig. 4b. The spermatic tissue of the treated animals at 500 mg/kg bwt showed less compact spermatogenic cells attaching to the sertoli cells (red arrowed). Mag. X400

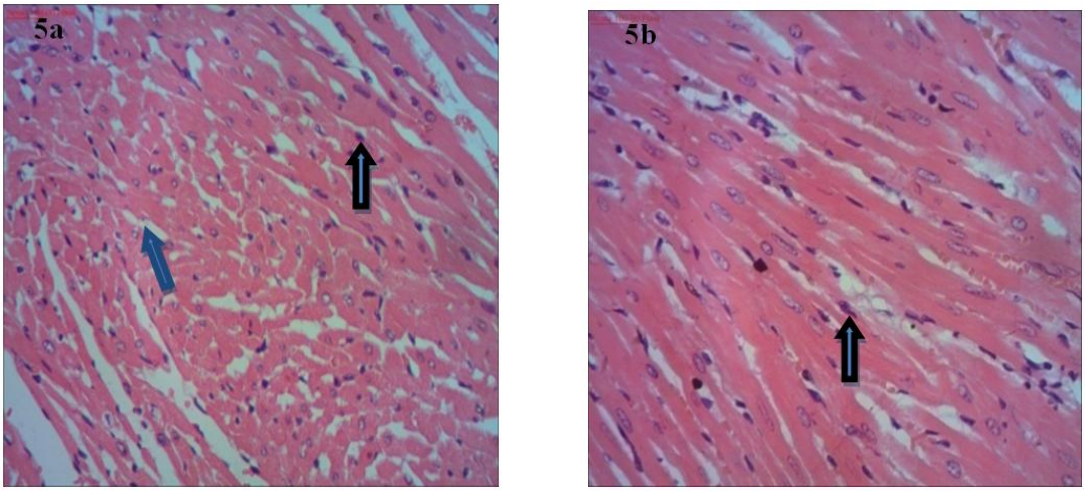


Fig. 5a. A section of myocardium (control) showing myocyte (black arrowed) separated by an unremarkable interstitium (blue arrowed). Fig. 5b. The cardiac tissue of the treated animals at 500 mg/kg bwt showed no pathological changes in the myocytes and at the interstices (black arrowed). Mag. X400

4. DISCUSSION

Global burden of infectious diseases caused by bacterial agents is a serious threat to public health [25]. Antibiotic treatment is a preferred choice to control bacterial infections; however, emergence of antimicrobial resistance and toxicity issues subside the use of antibiotic [26,27]. The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world as alternative to antibiotic [10]. Therefore experimental screening is important in order to establish the active principles as well as ascertain the efficacy and safety of herbal products [28]. This study revealed that ethanolic extracts of the root of *C. portoricensis* exhibited antibacterial activity against *E. facaelis* and *Strept. pneumonia* (ATCC 49619) at all the concentration (150 mg/ml, 300 mg/ml and 600 mg/ml) used. However, the extract exhibited no antibacterial activity against other bacterial strains (*Pseudomonas species* ATCC 27853, *Escherichia coli* ATCC 259218, *Staphylococcus aureus* ATCC 25923 *and Klebsiella species*) tested. In contrast, studies have shown that the leaves extract of *C. portoricensis* exhibited antibacterial activities against *E. coli*, and *Staphylococcus aureus* [6,29]. The anomaly observed in these studies could be attributed to the variation of bioactive compounds occurrence in different parts of the same plant or even in the same plant found in different environments [15], or to solvents and extraction methods employed for the extraction of the active constituents. Crude extract contains multiple active principles which could act singly or in combination (synergism) to bring about their antibacterial activities. The finding also gave credence to the folkloric uses and claims that the extract could be efficacious in the treatment of urinary tract infections and also support the use locally to treat women with infertility problems. The antibacterial activity of the extract against *S. facaelis* also implies that the root extract could be useful in the treatment of bacterial *endocarditis*. The MIC values of the extract was 6.4 mg/ml for *Streptococcus pneumonia* (ATCC 49619), and for *Streptococcus facaelis*, a higher a MIC value of 51.2 mg/ml was observed meaning that more of the extract will be needed to inhibit its growth. The extract demonstrated to have a low MBC value which was the same for both organisms.

Available information indicates that although some herbal medication have shown to be effective in the treatment of certain disease conditions, they have also exhibited harmful effect with marked deleterious changes on tissue morphology of some vital organs [15,30]. Experimental screening is therefore required to ascertain the safety of these drugs. According to World Health Organization (WHO), toxicity index of 2 g/kg bwt and above of a substance was considered safe [31]. The extract had LD_{50} above WHO toxicity index and therefore could be classified as being non toxic. The LD_{50} value of 5 g/kg bwt translates to 375 g dose equivalence in human adult and this is a very high value making the extract relatively safe for use. The viscera of the dead animals did not show any macroscopic changes that could point to the cause of the death neither did the animals convulse before dying showing that the extract did not kill the mice by the action on the nervous system [32]. The animals exhibited marked body weight decrease that was dose dependent. This could have resulted from the suppression of the appetite by the extracts and its constituents.

In this study, the organ morphology of the treated animals showed no colour changes compared to the control but histological studies revealed mild inflammatory changes in the liver. The hepatic tissue showed severe sinusoidal and portal congestions but with no necrotic damage. The liver and heart release AST and ALT, and an elevation in their plasma levels are usually reliable indices to inflammatory changes in both organs [18,19]. Therefore, the elevation that occurred in AST and ALT could be as a result of the observed inflammatory changes.

Creatinine is excreted by glomerular filtration and tubular secretion, the creatinine clearance is the rate at which creatinine is removed from the blood by the kidneys. The elevation in their levels indirectly suggests inefficiency or malfunction in renal filtration mechanism [18]. The photomicrograph of renal tissue of the treated animals showed normal renal corpuscles with also no abnormality at the interstices. Therefore, the increase in plasma creatinine and urea levels may be due to implicit inflammatory changes in the kidney. The photomicrograph of testicular tissue of the treated animals showed less compact spermatogenic cell mass with the interstices devoid of inflammation.

Serum bilirubin levels are reported as total bilirubin including both conjugated and unconjugated forms and there are three major causes. An increased bilirubin could be distinguished as haemolysis, biliary obstruction

and liver cell necrosis. However, a decrease in bilirubin level was recorded in the treated animals with the highest extract dose (500 mg/kg) group exhibiting marked decrease. It was evident there was no biliary obstruction and by implication the sinusoidal congestion observed in the hepatic tissue did not affect the biliary system. The study showed a marked increase in total protein level. An increase in total protein level has been reported to have hepato-protective effect on the cells [3,33].

The reduction in the LDL- cholesterol in all the treated rats is an indication that the extract can reduce the cardiovascular risk factors which contribute to death of diabetic subjects [34]. Meanwhile, the HDL increased only in 250 mg/kg bwt extract dose. An increase in the HDL level is a favourable attribute of the extract. This is mainly because HDL is the good cholesterol and is needed in certain amount by the body.

The RBC levels showed decrease at the highest dose treatment while at the lower doses increased marginally compared to the control. In this context, the possibility that the extract does have the potential to stimulate erythropoientin release in the kidney was unlikely. The decrease which occurred in Hb levels indicated the likely occurrence of haemolytic anaemia and polycythemia. The white blood cells serve as scavengers that destroy the microorganisms at infection sites, removing foreign substances and debris that results from dead or injured cells [35]. The appreciable increase observed in the WBC levels of all treated animals could be attributable to the mobilization of body defense system in response to toxic environment [36].

The values of RBC indices fluctuated, while those for MCV increased except for the highest extract dose, MCHC and MCH respectively showed decrease respectively. The RBC indices are known to be of unique importance in amaenia diagnosis in most animals [37]. Low MCHC is associated with iron deficiency anemia where microcytic, hypochromic red cells are produced as a result of lack of iron to support haemoglobin synthesis [38]. Therefore, the plant might possess the potential to induce microcytic anaemia as have been observed in some plants [30].

5. CONCLUSION

The extract exhibited have high safety margin since the LD_{50} was 5.0 g/kg bwt. The study revealed that *C. portoricensis* could be an effective antibacterial agent possessing bactericidal activity against some *Streptococcus pneumonia* and *Streptococcus facaelis*. However, the hepatic tissue morphology indicated the occurrence of inflammatory changes in 500 mg/kg bwt dose of the extract which showed sinusoidal and portal congestions.

CONSENT

This was not applicable since the study was on animals and not on humans.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" [39] and ethical guidelines for investigation of experimental pain in conscious animals [40] were strictly followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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