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Research article

# *In vivo* anti-typhoid and safety evaluation of extracts of *Ximenia americana* on experimental rats

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ABSTRACT

*Background:* Typhoid fever is an infectious disease of serious public health concern. The anti-typhoid efficacy and safety of *Ximenia americana* stem bark extracts on the nephrocytes, hepatocytes, and haematological parameters of *Salmonella typhi* infected Wistar albino rats was evaluated.

*Methodology:* Experimental rats in their respective groups were infected with *S. typhi* via oral administration of infective dose (0.5 ml of $1.5 \times 10^4$ cfu/ml) of cultured *S. typhi,* while group 1 were uninfected. Group 2 were untreated, group 3 were treated with the standard drug (ciprofloxacin), while groups 4, 5, 6 were treated with (100, 200, 300 mg/kg) body weight of the methanol extract of *X. americana* stem bark, and groups 7, 8 and 9 were treated with (100, 200, 300 mg/kg) body weight of the ethyl-acetate fraction of *X. americana* stem bark administered orally for 7 days. Acute toxicity test was carried out using standard methods and signs accompanying toxicity such as drowsiness, respiratory distress, diarrhoea, and possible death of animals were monitored for 24 h in an interval of 2 h. Physical parameters were also monitored to check symptom amelioration as the treatment progresses.

*Results:* Acute toxicity test showed that experimental rats administered the methanol extract and ethyl-acetate fraction of *X. americana* had no clinical signs of toxicity such as drowsiness, respiratory distress, diarrhoea, and death. The Physical examination of rats in all the treatment groups showed improvement in symptoms following *Salmonella* infection as treatment progressed when compared to the untreated group. *Salmonella* infection led to a significant ($p < 0.05$) increase in serum urea, sodium, chloride levels, bilirubin concentration, total white cell count and activities of the liver enzymes as compared to normal and apparently healthy rats. A significant ($p < 0.05$) decrease in PCV, haemoglobin concentration, RBC count, serum potassium, total protein and creatinine levels of the infected groups was observed as compared to the normal uninfected group. The methanol extract at the dose of 300 mg/kg body weight had 15%, 37%, 52% reduction in sodium, urea, and alkaline phosphatase respectively and 57% increase in potassium while the ethyl-acetate fraction (300 mg/kg body weight) had 46% and 47% increase in PCV and haemoglobin and 36% reduction in ALT.

*Conclusion:* The administration of the methanol extract and ethyl-acetate fraction of *X. americana* was accompanied by a significant ($p < 0.05$) dose dependent decrease in serum electrolytes and alkaline phosphatase in the treated groups. *X. americana* could be a safe natural alternative therapeutic source for enteric fever management.

## 1. Introduction

Enteric fever also called (typhoid fever) or typhoid is a serious bacterial bloodstream infection caused by bacterial pathogen *Salmonella typhi* (*S. typhi*) and *Para typhi* A, is an important cause of morbidity and mortality in the developing world. Transmission occurs faeco-orally via contaminated water and food. An estimated 14.3 million infections and more than 135,000 deaths are caused by enteric fever worldwide each year [1]. Enteric infection affects children and young adults most frequently than the old adults. Signs and symptoms of the infection includes mostly abdominal pain, anorexia, constipation, diarrhoea, fever, frontal throbbing headache, gastrointestinal bleeding, hepatosplenomegaly, and nausea [2]. *S. typhi* has the capability of affecting

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almost every organ system thus, consequently those infected are susceptible to a widespread variety of complications. Amongst these complications intestinal perforation occurs in 1–3% of cases, and it is associated with the highest mortality [3,4,5]. Perforations can occur at any site from the duodenum to the colon, though the ileum is the most common site. Gall bladder perforation has also been reported. In young children, several perforations are often extant. Other complications include heart failure from myocarditis or endocarditis, liver failure from hepatitis or pancreatitis, renal failure from pyelonephritis or glomerulonephritis and respiratory failure from pneumonia, as well as disseminated intravascular coagulation, arthritis and orchitis [3]. Effective treatment in addition to improving drinking water and sanitation is required to reduce the burden of typhoid fever. Presently, World Health Organization [6] recommends the use of third-generation cephalosporines and azithromycin for its management. The microbial agents have however developed resistance and thus threaten the effectiveness of most of these antimicrobials [7]. More so, antimicrobial drug resistance is widespread, and patients treated with ineffective antimicrobials show a poor clinical response and a higher rate of complications and deaths, as well as prolonged shedding of the pathogen in stool, which sustains transmission and induces secondary cases [8]. As a result, alternative agents from natural products with therapeutic potentials and mild adverse effects are sought for.

*Ximenia americana* also known as wild olive is a shrub-like plant that grows widely in humid and temperate regions of the world. It is extensively used as an herbal medicine for treating malaria, leprotic ulcers, and skin infections amongst the Hausa/Fulani communities of Northern Nigeria [9]. Various extracts (including methanol, ethanol, chloroform, ethyl-acetate, aqueous and petroleum ether) from the plant have been reported to have antibacterial, analgesic, antiviral, and antitrypanosomal properties [10,11,12,13,14]. In addition they contain tannins, flavonoids, alkaloids, saponins, anthraquinones, starch, glycosides, which are presumed to be responsible for their various biological activities. This study therefore aims to investigate the efficacy of *X. americana* stem bark extracts as an antibacterial agent and its adverse effect (if any), on the nephrocytes, hepatocytes, and haematological parameters.

## 2. Materials and methods

### 2.1. Plant material

*Ximenia americana* stem bark was collected from the host plant in February 2020 in Bosso town Minna, Nigeria. The plant was identified at the herbarium section of Plant Biology Department, Federal University of Technology Minna, Nigeria and authenticated at the Herbarium Unit, National Institute for Pharmaceutical Research and Development Abuja Nigeria, where voucher specimen was deposited with number NIPRD/H/7247.

### 2.2. Experimental animals

A total of forty-eight (48) disease-free Wistar albino rats weighing (140–200 g) were purchased from Odata farm in Ilorin, Kwara State. They were kept in clean plastic cages with wood shavings as beddings and conveniently housed in the animal house of Biochemistry Department, Federal University of Technology Minna, Nigeria under standard environmental conditions of temperature (27–29 °C), 70% relative humidity, 12-hour light/night cycle. They were fed with a diet of grower's mash and adequate water ad libitum.

#### 2.2.1. Ethics statement

The animals were allowed to acclimatize to laboratory environment for two weeks before initiation of the experiment and the standard protocol under the Canadian Council on Animal Care Guidelines and Protocol Review [15] was observed as adopted and approved by the ethics committee on animal handling and experimental protocol of Federal University of Technology Minna, Nigeria.

### 2.3. Test bacteria

Stock culture of test bacteria (*S. typhi*) used in the study was obtained from the vaccine production laboratory in Africa Centre of Excellence for Mycotoxin and Food Safety, Federal University of Technology Minna. *S. typhi* was confirmed by carrying out gram's staining, culturing on appropriate selective media, and subjecting it to various biochemical tests.

### 2.4. Extraction and phytochemical analysis

#### 2.4.1. Preparation of plant extract and fractionation

The stem bark of *X. americana* was air-dried in the Laboratory of the Department of Biochemistry for two weeks and the dried samples were crushed using mortar and pestle and milled to powder with electric blender. Fifty grams (50 g) of the powdered sample was extracted under reflux in 300 ml of methanol for 2 h by the method of [16]. The extract was filtered hot using muslin cloth and the solvent was removed by rotary evaporator. The extract obtained was transferred into a sterile universal bottle and kept in the refrigerator until required for use. The ethyl-acetate fraction was obtained according to [9] by dissolving 0.5 g of methanol extract in 50 ml of water and the mixture was waggled several times with 100 ml of ethyl-acetate using a separating funnel. The ethyl-acetate layer collected was combined and evaporated to obtain the fraction.

#### 2.4.2. Qualitative estimation of secondary metabolites of methanol extract and ethyl-acetate fraction of X. americana stem bark

The qualitative phytochemical analysis of the crude extract and fraction of *X. americana* to identify the presence of secondary metabolites: tannins, phenolic compounds, steroids, anthraquinones, saponins, flavonoids and alkaloids were carried out according to standard methods of [17,18].

#### 2.4.3. Quantitative estimation of secondary metabolites of methanol extract and ethyl-acetate fraction of X. americana stem bark

Secondary metabolites in both extracts were quantified using standard procedures as described by [19] for total phenols, [20] for flavonoids, [21] for total alkaloids, [22] for saponins, and [23] for tannins.

### 2.5. Acute oral toxicity study (safe dose determination)

Acute toxicity study of the crude methanol plant extract was carried out using Wistar albino rats of both sexes adopting the guideline of the organization for economic corporation and development standards [24]. Lorke's method [25] was used to study the toxicity effect of the methanol extract and the ethyl-acetate fraction of *X. americana* in Wistar albino rats. The study involves the oral administration of different doses of both extracts to 36 rats of 12 groups consisting of 3 animals each of different sexes. Labeled rats in the first six groups were respectively administered the extract and fraction orally at graded doses of 10, 100, 1000 mg/kg body weight (phase I). Similarly, the last labeled six groups were administered respective extract and fraction orally at graded doses of 1600, 2900, and 5000 mg/kg body weight in the second stage (phase II). Signs accompanying toxicity such as drowsiness, respiratory distress, diarrhoea, and possible death of animals were monitored for 24 h in an interval of 2 h and the safe dose was established to be up to 5000 mg/kg body weight.

### 2.6. Experimental design and establishment of Salmonella typhi infection

The animals were allowed to acclimatize in the animal house of the Department of Biochemistry Federal University of Technology Minna, Nigeria for two weeks. They were thereafter randomly allotted to nine cages consisting of three rats. The experiment was conducted for seven days and the extract and fraction were administered orally.

Group 1: Positive control administered normal saline (Not infected, not treated)

Group 2: Negative control, infected with *S. typhi* but not treated

Groups 3, 4, 5, 6, 7, 8 and 9: Infected with *S. typhi* and treated orally with standard drug (Ciprofloxacin), 100, 200, 300 mg/kg body weight of the crude methanol extract, 100, 200, and 300 mg/kg body weight of the ethyl-acetate fraction respectively.

### 2.7. Infection procedure and establishment of S. typhi infection

Animals were screened bacteriologically for *S. typhi* infection [26] and thereafter starved overnight before the bacterial inoculation. Rats in groups 2, 3, 4, 5, 6, 7, 8, and 9 were orally administered 0.5 ml of $1.5 \times 10^4$ cfu/ml of the cultured *S. typhi* infective dose. Some of the symptoms observed include unformed stool, loss of appetite, general body weakness, salivation, scattered fur, falling of hairs, stool with mucous and weight loss. One rat from each group that was infected with *S. typhi* was picked at random and the blood samples were cultured using *Salmonella-Shigella* Agar (SSA) and incubated (24 h) for bacterial growth.

### 2.8. Blood sample collection, serum preparation and analysis

#### 2.8.1. Blood sample collection and serum preparation

Collection of serum for biochemical analysis was carried out as described by [27]. Twelve hours after the final treatment, the rats were anesthetized under diethyl ether, euthanized and the blood samples were collected by jugular puncture. The blood samples for kidney and liver function tests were collected into dry tubes and allowed to stand at room temperature for 15 min and then centrifuged at 1500 rpm/10 min. While the blood samples for the haematological analysis were collected in K2 EDTA containers.

#### 2.8.2. Biochemical analysis

Biochemical parameters analysed were serum electrolytes (sodium, chloride, and potassium), serum urea and creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, and bilirubin (total and direct) using a semi-auto chemistry analyser (Rayto RT-9200 China).

#### 2.8.3. Haematological analysis

Haematological parameters analysed include: haemoglobin concentration (Hb), Parked cell volume (PCV), Red blood cell (RBC) count, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean haemoglobin concentration (MCH), red cell distribution width (RDWC), total white blood cell count (TWBC), lymphocyte, neutrophils, monocytes, eosinophils, and basophils were determined using an automated blood analyser (Accu cell DX 360 China).

### 2.9. Statistical analysis

Data collected from the analysis were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20.0 windows version. Results were presented as mean ± standard error. Mean with differences were separated using Duncan Multiple Range Test and results were considered significant at $P < 0.05$.

**Table 1**
Quantitative composition of secondary metabolites of *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction.

| PHYTOCHEMICAL (mg/g) | ME | EF |
|---|---|---|
| Total phenols | 101.09±0.05 | 89.94±0.08 |
| Flavonoids | 15.15±0.02 | 10.10±0.05 |
| Alkaloids | 257.65±0.33 | 193.35±0.41 |
| Saponins | 36.75±0.01 | 30.45±0.03 |
| Tannins | 105.74± 0.04 | 98.86±0.08 |

Values are mean ± standard error of three replicates ($n = 3$).
KEY: ME- Methanol extract; EF- Ethyl-acetate fraction

## 3. Results

### 3.1. Qualitative estimation of secondary metabolites of the Ximenia americana stem bark methanol extract and ethyl-acetate fraction

Results of the qualitative analysis of the extract and fraction revealed the presence of alkaloids, tannins, saponins, phenols, flavonoids, terpenes, while anthraquinones and phlobatannins were present only in the methanol extract.

### 3.2. Quantitative estimation of secondary metabolites of Ximenia americana stem bark methanol extract and ethyl-acetate fraction

The quantitative phytochemical analysis (Table 1) showed the extract and fraction contain significant concentration of alkaloids, tannins, and total phenols. Alkaloid was particularly the highest in both methanol extract and ethyl-acetate fraction (257.65±0.33 and 193.35±0.41 mg/g) respectively. Other phytochemical found in substantial amounts in descending order in the extract and fraction are tannins, total phenols, saponins, and flavonoids

### 3.3. Acute oral toxicity study

No toxicity signs (drowsiness, respiratory distress, and diarrhoea) or death were recorded following administration of the extract nor its fraction, indicating that the safe dose of both the methanol extract and its ethyl-acetate fraction of *Ximenia americana* stem bark was up to 5000 mg/kg body weight.

### 3.4. Effect of Ximenia americana (methanol extract and ethyl-acetate fraction) on the physical parameters of S. typhi infected rats

Two days after extract and fraction administration, the animals had improved formation of stool without mucus, food intake improved, the fall in hair ceased gradually and this progressed throughout the treatment period.

### 3.5. Effect of Ximenia americana stem bark methanol extract and ethyl-acetate fraction on kidney function parameters of S. typhi infected rats

Effect of both the methanol extract and the ethyl-acetate fraction of *X. americana* stem bark on the kidney function parameters of the rats are presented in Table 3. Serum urea (21.00±8.00 mmol/L), sodium (87.50±2.50 mmol/L), and chloride levels (88.50±10.50 mmol/L) were significantly ($p < 0.05$) increased in groups infected with *S. typhi* when compared to the positive control (13.33±0.88 mmol/L, 73.33±11.70 mmol/L and 80.67±7.31 mmol/L for urea, sodium and chloride respectively). Serum potassium (0.59±1.00 mmol/L) and creatinine (0.60±0.10 mg/dL) levels reduced significantly ($p < 0.05$) in the group infected with *S. typhi* when compared to the positive control (1.90±0.03 mmol/L and 0.83±0.15 mg/dL for potassium and creatinine respectively) and the treatment groups. There was a dose dependent decrease in the concentration of serum urea, sodium, and

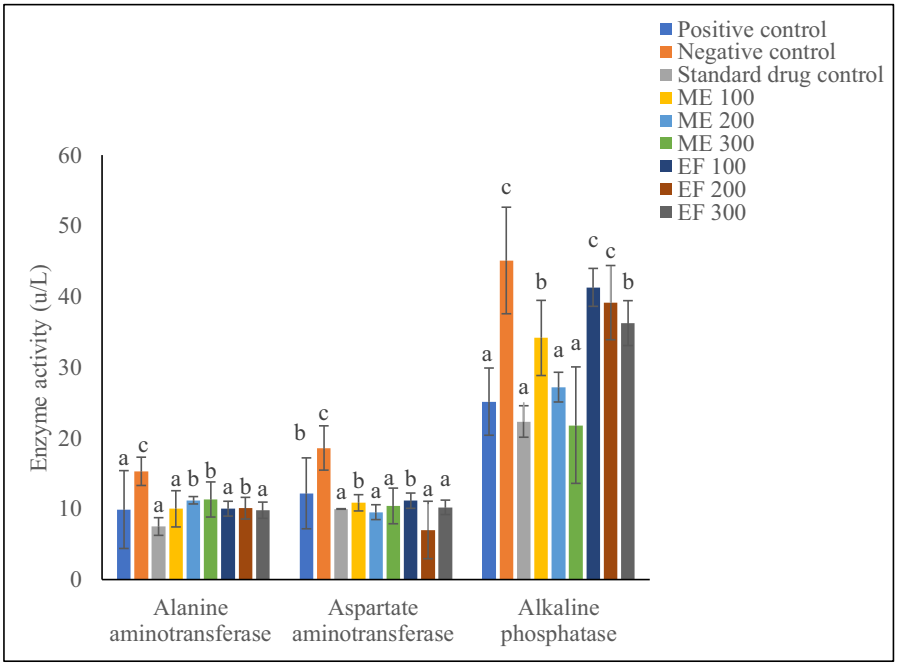


**Fig. 1.** Effect of *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction on the liver enzymes. Key: ME-methanol extract, EF-ethyl-acetate fraction (Doses are in mg/kg body weight). Values are mean ± standard error of three replicates ($n = 3$). Columns with different alphabets for each parameter are significantly different ($p < 0.05$).

chloride as well as a dose dependent increase in potassium and creatinine in the treated groups. Comparing the extract treated groups with the standard drug control group as shown in Table 3, no significant difference was observed in the groups treated with 200 and 300 mg/kg body weight of the methanol extract (77.00±0.58 mmol/L and 74.00±3.79 mmol/L) and 100 mg/kg body weight of the ethyl acetate fraction (80.00±9.17 mmol/L) when compared to the standard drug group (75.33±3.18 mmol/L) for sodium, the group treated with 300 mg/kg body weight of the two extracts (1.38±0.29 mmol/L and 1.20±0.21 mmol/L) were comparable to the standard drug control group (1.56±0.08 mmol/L) for potassium. Chloride concentration for all the treated groups were comparable to the standard drug group. Groups treated with 200 and 300 mg/kg body weight methanol extract (14.83±0.70 mmol/L and 13.33±1.45 mmol/L) and all the ethyl-acetate fraction treated groups had their values comparable to the standard drug control group (13.53±0.26 mmol/L) for urea. Creatinine concentration in groups treated with the both extracts (200 and 300 mg/kg body weight) were having no significant difference when compared to the standard drug control group.

### 3.6. Effect of Ximenia americana stem bark methanol extract and ethyl-acetate fraction on liver enzymes of S. typhi infected rats

Effect of both methanol extract and ethyl-acetate fraction of *X. americana* stem bark on the liver enzymes of the rats are presented in Fig. 1. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in the *Salmonella* infected but not treated rats (negative control) was significantly ($p < 0.05$) increased (15.30±2.00 u/L; 18.60±3.13 u/L; 45.10±7.53 u/L) respectively, compared to the positive control group (9.90±5.50 u/L; 12.19±5.01 u/L; 25.15±4.75 u/L). However, daily administration of extract and fraction to infected rats at the stated doses significantly ($p < 0.05$) decreased the activities of the liver enzymes. The reduction observed for ALP was dose dependent in both extract and fraction, while the reduction observed for AST and ALT were significant but dose independent with doses 200 and 300 mg/kg body weight of ethyl-acetate fraction having the highest reduction (7.01±4.06 u/L and 9.80±1.15 u/L). From Fig. 1 below, the AST values obtained for all the extract treated groups except the group treated with 200 mg/kg body weight were having no significant difference when compared to the standard drug control group. In addition, the ALP value obtained for the group treated with 300 mg/kg body weight of the methanol extract (21.20±8.26 u/L) was comparable to the standard drug control group (22.32±2.25 u/L).

### 3.7. Effect of Ximenia americana stem bark methanol extract and ethyl-acetate fraction on serum bilirubin concentration of S. typhi infected rats

In Fig. 2, all the treatment groups had a significant reduction in both total and direct bilirubin concentrations with the group treated with 300 mg/kg body weight of the methanol extract having the highest reduction (2.30±1.58 µmol/L; 1.30±0.10 µmol/L) when compared to the negative control (8.50±1.20 µmol/L; 5.10±0.10 µmol/L).

### 3.8. Effect of Ximenia americana stem bark extract and ethyl-acetate fraction on serum total protein, albumin, and some haematological parameters of S. typhi infected rats

Fig. 3 shows the effect of both the methanol extract and the ethyl-acetate fraction of *X. americana* stem bark on total protein, albumin, and some red blood cell parameters of the rats. Induction of *S. typhi* infection resulted in significant ($p < 0.05$) reduction in total protein, haemoglobin concentration, and mean cell haemoglobin concentration (6.10±0.98 g/dL, 7.21±0.66 g/dL and 23.97±1.45 g/dL) respectively as compared with the positive control that had (7.99±0.05 g/dL, 12.00±0.06 g/dL, 33.81±1.53 g/dL) and non-significant ($p>0.05$) decrease in albumin concentration (2.20±0.02 g/dL) in the negative control group when compared to the positive control group (2.48±0.10 g/dL). The treated groups had significant ($p < 0.05$) dose independent increase in the concentration of the parameters tested except for haemoglobin, with some treated groups been comparable to the positive control particularly the groups treated with 200 and 300 mg/kg body weight of the ethyl-acetate fraction for total protein (8.05±1.56 g/dL) and haemoglobin concentration (13.65±2.52 g/dL). So also, the group treated with 200 mg/kg body weight of the methanol extract for mean cell haemoglobin concentration (34.65±2.67 g/dL). Increase in haemoglobin concentration for the groups treated with

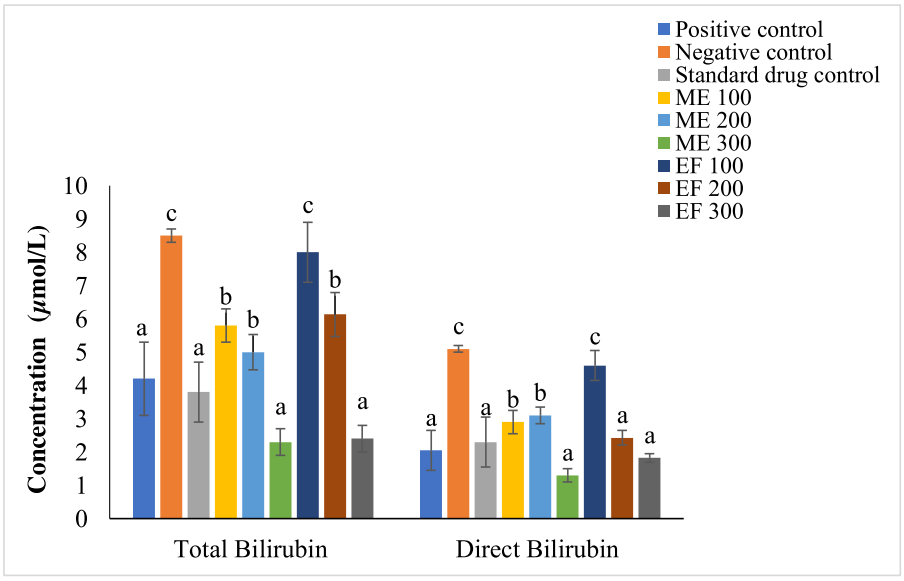


**Fig. 2.** The effect of *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction on serum bilirubin concentration. Key: ME-methanol extract, EF-ethyl-acetate fraction (Doses are in mg/kg body weight). Columns with different alphabets for each parameter are significantly different ($p < 0.05$).

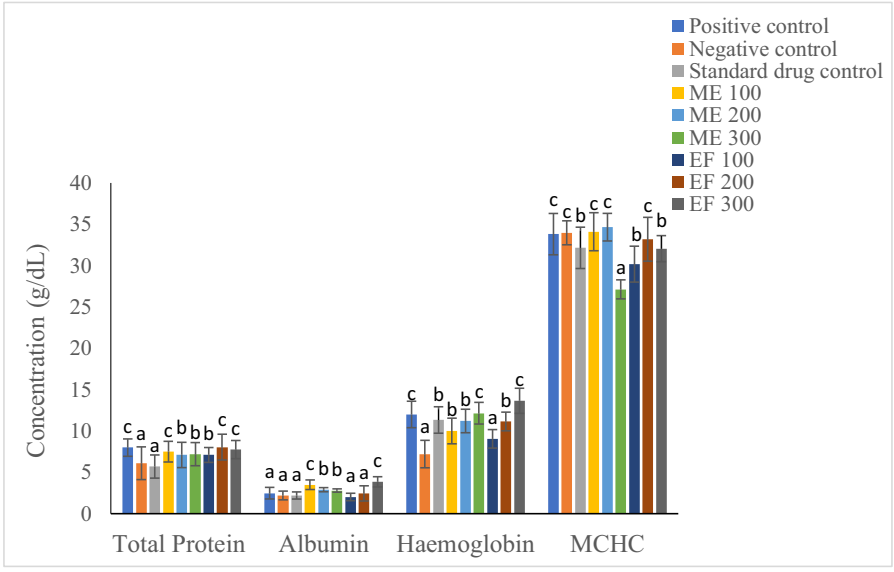


**Fig. 3.** The effect of *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction on total protein, albumin, and some red blood cell indices. Key: ME-methanol extract, EF-ethyl-acetate fraction (Doses are in mg/kg body weight), MCHC-mean cell haemoglobin concentration. Columns with different alphabets for each parameter are significantly different ($p < 0.05$).

200 mg/kg body weight of the methanol extract and ethyl-acetate fraction had values (11.21±1.42 g/dL and 11.15±1.13 g/dL) respectively, which have no significant difference when compared to value for the standard control group (11.33±1.60 g/dL).

### *3.9. Effect of Ximenia americana stem bark extract and ethyl-acetate fraction on the red blood cell count of S. typhi infected rats*

Fig. 4 shows that rats in the treated groups had significant increase in red blood cell count in a dose dependent manner when compared to the negative control. All the treated groups had values that are comparable to the value for the standard drug control group except the group treated with 100 mg/kg body weight of the ethyl-acetate fraction.

### *3.10. Effect of Ximenia americana stem bark methanol extract and ethyl-acetate fraction on the mean cell haemoglobin of S. typhi infected rats*

In Fig. 5, the various treatment groups had a significant increase in mean cell haemoglobin in a dose independent manner when compared to the negative control (15.93±1.28 pg), with the group treated with 300 mg/kg body weight of the methanol extract having the highest (26.18±1.52 pg).

### *3.11. Effect of Ximenia americana methanol extract and ethyl-acetate fraction on the mean cell volume of S. typhi infected rats*

In Fig. 6, all the treatment groups had a significant reduction in mean cell volume with the group treated with 300 mg/kg body weight of the ethyl-acetate fraction having the highest reduction (48.02±6.21 fl), when compared to the negative control (75.80±5.69 fl). However, decrease in mean cell volume in the treated group was dose independent.

### *3.12. Effect of Ximenia americana stem bark methanol extract and ethyl-acetate fraction on differential white blood cell count and packed cell volume of S. typhi infected rats*

Fig. 7 shows the effect of both the methanol extract and the ethyl-acetate fraction of *X. americana* stem bark on the white blood cell parameters and packed cell volume of the rats. Induction of *S. ty-*

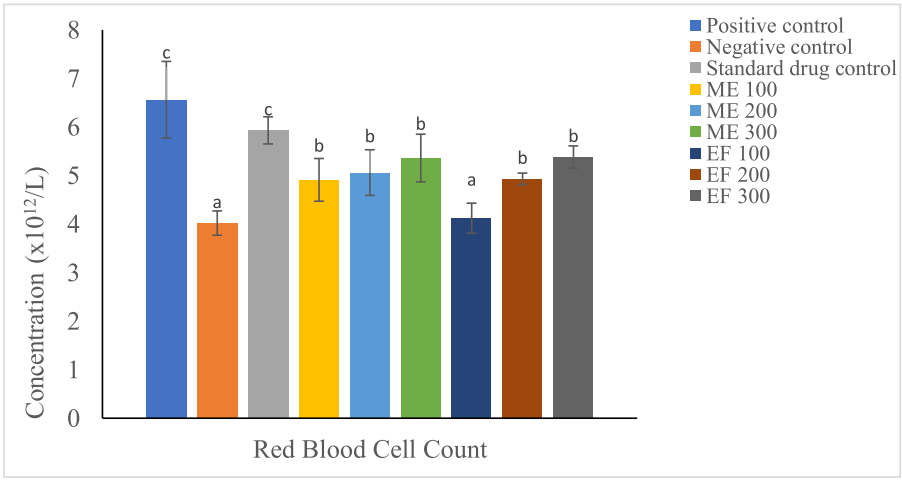


**Fig. 4.** The effect of the *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction on red blood cell count. Key: ME-methanol extract, EF-ethyl-acetate fraction (Doses are in mg/kg body weight). Columns with different alphabets are significantly different ($p < 0.05$).

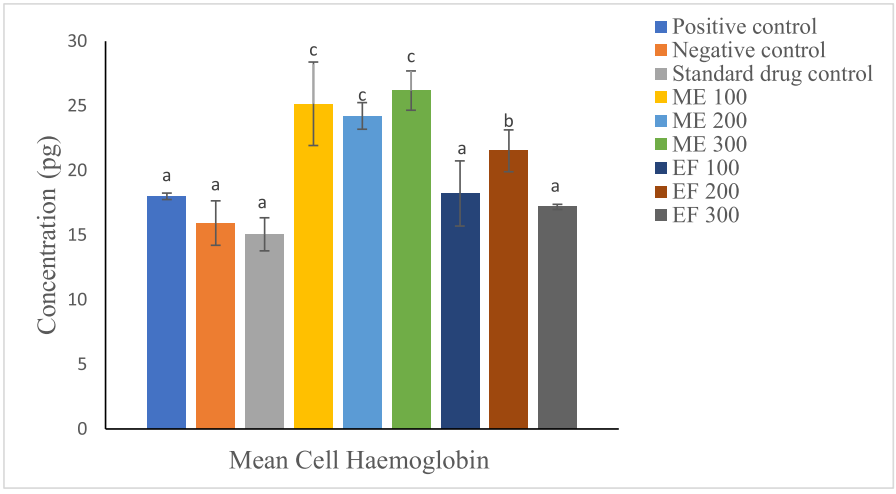


**Fig. 5.** The effect of the *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction on mean cell haemoglobin Key: ME-methanol extract, EF-ethyl-acetate fraction (Doses are in mg/kg body weight). Columns with different alphabets are significantly different ($p < 0.05$).

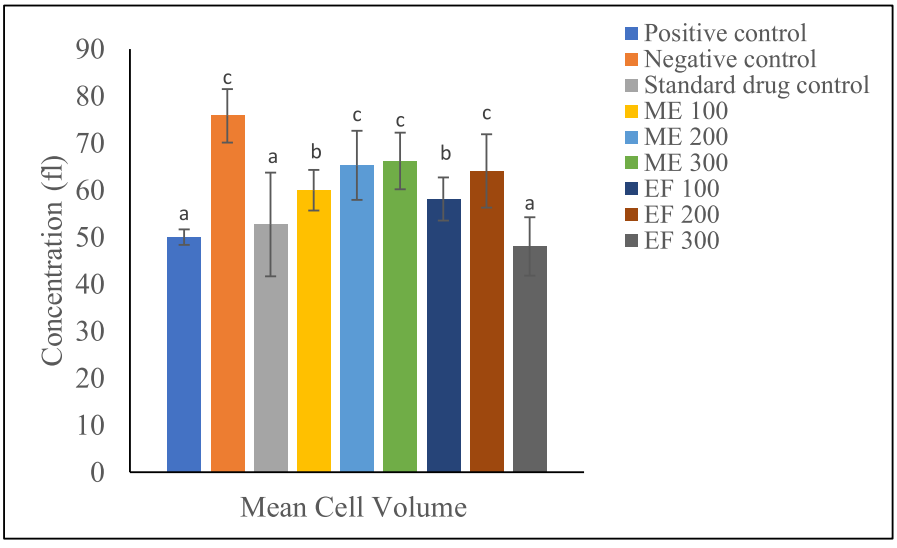


**Fig. 6.** The effect of *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction on mean cell volume. Key: ME-methanol extract, EF-ethyl-acetate fraction (Doses are in mg/kg body weight). Columns with different alphabets are significantly different ($p < 0.05$).

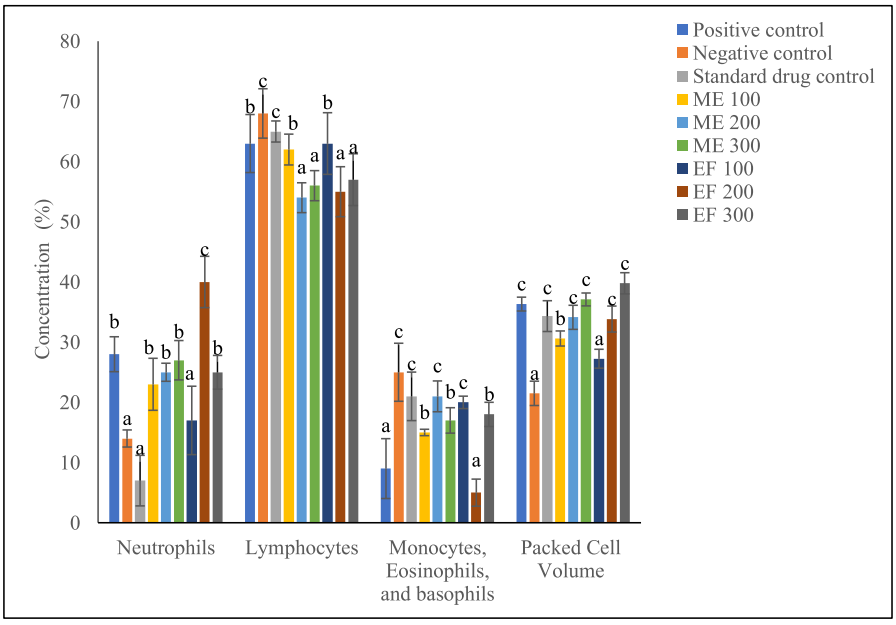


**Fig. 7.** The effect of *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction on differential white cell count and packed cell volume. Key: ME-methanol extract, EF-ethyl-acetate fraction (Doses are in mg/kg body weight). Columns with different alphabets for each parameter are significantly different ($p < 0.05$).

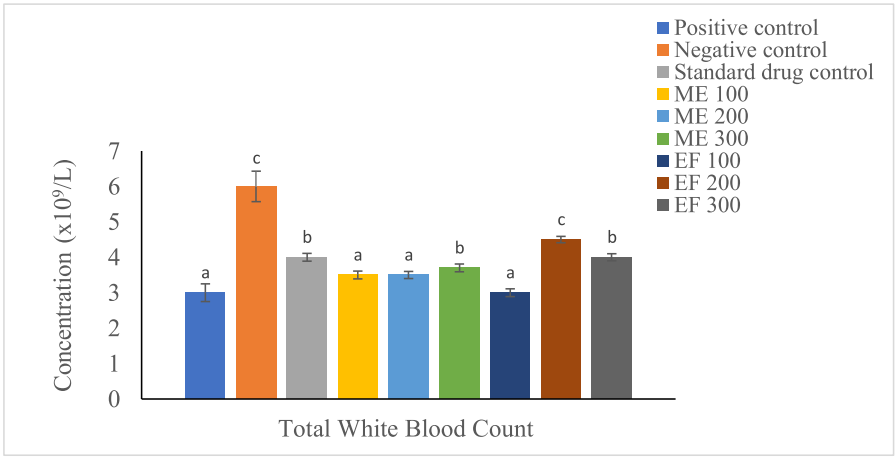


**Fig. 8.** The effect of *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction on total white blood cell count. Key: ME-methanol extract, EF-ethyl-acetate fraction (Doses are in mg/kg body weight). Columns with different alphabets are significantly different ($p < 0.05$).

*phi* infection resulted in significant ($p < 0.05$) reduction in neutrophil (14.0±1.42%) and packed cell volume (21.5±2.56%) as compared to the positive control (28.0±2.90% and 36.33±1.15%) while monocyte, eosinophil & basophil (25.0±4.82%) and lymphocytes (68.0±4.10%) increased in all groups as compared to the positive control (09.00±4.97% and 63.0±4.82%) respectively. In the treated groups, significant increase in neutrophil and packed cell volume was observed particularly in the groups treated with 200 and 300 mg/kg body weight of the ethyl-acetate fraction (40.0±4.26% and 39.78±0.76%) respectively. Same significant reduction in the count of monocyte, eosinophil and basophil was observed in the group treated with 200 mg/kg body weight of the ethyl-acetate fraction (5.0±2.25%). Similarly, highest reduction was observed in lymphocyte count in the group treated with 200 mg/kg body weight of the methanol extract (54.0±2.4%).

### *3.13. Effect of Ximenia americana stem bark extract and ethyl-acetate fraction on the total white blood cell count of S. typhi infected rats*

In Fig. 8, the total white blood cell count for the treatment group was reduced in a dose independent manner with the dose treated with 100 mg/kg body weight of the ethyl-acetate fraction having the highest reduction ($3.0 \pm 0.11 \times 10^{9}$/L) when compared to the negative control ($6.0 \pm 0.43 \times 10^{9}$/L).

## 4. Discussion

The application of medicinal plants in the management and treatment of various illnesses is very rampant all over the world with Nigeria not an exception. Plants have been used for the management of a wide range of diseases for a long time in form of decoction and infusion, and this may be due to the presence of a variety of secondary metabolites that are said to be responsible for their medicinal properties. *Ximenia americana* is a plant used in traditional medicine for the treatment of skin infections, ulcers, and leproitic lesions in Northern Nigeria [14]. Although traditional medicine practitioners have appraised its effectiveness, there is no documented scientific evidence to support its safety and use for the treatment of typhoid/enteric fever nor other infections caused by various microbial organisms. This study tends to provide scientific proof for the use of *X. americana* as a remedy for enteric fever as well as search for the preliminary toxicological profile that may guide the rural community users. Secondary metabolites present in most plants are responsible for the medicinal potentials they exhibit. Qualitative phytochemical analysis shows that the methanol extract of *X. americana* stem bark contains secondary metabolites such as saponins, phenols, flavonoids, alkaloids, phlobatannins, anthraquinones, tannins, and terpenes, while the ethyl-acetate fraction contains alkaloids, saponins, tannins, phenols, terpenes, and flavonoids. This is consistent with the

**Table 2**
Physical parameter of treated and untreated rats.

| Days | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Positive control | k | k | k | k | k | k | k |
| Negative control | a, b, d, e | a, b, d, f, e | a, b, d, f, e | a, b, d, f, | a, b, d, f, | a, b, d, f, | a, b, d, f, |
| Standard control | a, b, d, e | a, b, d, f | a, b, d, f | c, g, h, j | c, g, h, j | c, h, i, j | h, i, j |
| ME 100 | a, b, d, e | a, b, d, f | a, b, d, f | a, b, c, f | c, g, h, j | g, h, j | h, i, j |
| ME 200 | a, b, d, e | a, b, d, f | a, b, d, g | c, f, h, j | c, g, h, j | h, i, j | h, i, j |
| ME 300 | a, b, d, e | a, b, d, f | a, b, d, f | c, f, h, j | c, g, h, j | h, i, j | h, i, j |
| EF 100 | a, b, d, e | a, b, d, f | a, b, d, f | a, c, f, j | c, g, h, j | g, h, j | h, i, j |
| EF 200 | a, b, d, e | a, b, d, f | a, b, d, f | c, g, h, j | c, g, h, j | g, h, j | h, i, j |
| EF 300 | a, b, d, e | a, b, d, f | a, b, d, f | c, g, h, j | c, g, h, j | h, i, j | h, i, j |

KEY: ME- Methanol extract; EF- Ethyl-acetate fraction (Doses in mg/kg body weight).
[a] Reduced activity.
[b] reduced food intake.
[c] Scattered fur.
[d] Scattered and falling fur.
[e] Drooling.
[f] Loose stool.
[g] Soft stool.
[h] Active.
[i] Formed stool.
[j] Improved food intake.
[k] Normal.

report of [28] but with volatile oils, cardiac and glycosides and resins in ethanol extract. In a study by [9,14], on the antimicrobial activity and constituents of ethanol and methanol extracts of *X. americana,* they asserted that the extracts contain no alkaloids and anthraquinone which disagrees with this study. The reason for the discrepancy could be variations in geographical locations of the plant materials, extraction methods and probably the adaptability of each plant species.

Further quantitative analysis of the extract and fraction (Table 1) showed that they both contain a significant concentration of alkaloids which have been reported to have a wide range of antimicrobial and insecticidal properties elicited by inhibiting DNA topoisomerase of microbes [29]. Other secondary metabolites found in substantial amount in the extract and fraction which could be responsible for their therapeutic effect on *S. typhi* infected rats are flavonoids, total phenols, and saponins (Table 1). Flavonoids and total phenols have been reported [30] to complex with extracellular and soluble proteins as well as with bacterial cell walls while tannins are capable of inactivating microbial adhesions, enzymes, cell envelope of transport proteins and also form complex with polysaccharide [31]. The safe dose for both extracts (methanol crude and ethyl-acetate fraction) after acute toxicological studies, was established to be >5000 mg/kg body weight. This is to say that the plant extracts have no clinical signs of toxicity (drowsiness, respiratory distress, and diarrhoea) in assays tested in this study and may therefore be safe at tested doses, though histopathological studies have not been carried out to confirm this.

The clinical signs observed upon the physical examination of the rats in each group (Groups 1–9) in this study are shown in Table 2, the rats in the negative control group were observed to have loose stool with mucus, reduced activity, loss of appetite, fur scattering and falling contrary to the animals in the positive control group with none of these signs. According to [32] most of these observations are signs of fever and diarrhoea. Animals in the extract treated groups after two days of treatment had their symptoms ameliorating as treatment progressed for 7 days. This result is in conformity with the works of [32] and [33] that both reported the disappearance of signs and symptoms after seven days of treatment in their separate studies.

The kidneys are the primary excretory organs responsible for electrolyte and fluid volume balance. The efficiency of the functional integrity of the kidney can be gauged by measuring the serum concentration of biomarkers such as serum urea, creatinine, and electrolytes (sodium, potassium, and chloride). In this study, the kidney function parameters for the entire experimental groups were evaluated. Serum urea, sodium, and chloride levels increased significantly ($p < 0.05$) in the negative control group compared to the positive control (Table 3). Serum potassium and creatinine levels reduced significantly ($p < 0.05$) in the negative control group when compared to the positive control and the treatment groups (Table 3). This is complementary to the work of [34] who reported same for serum urea, potassium, and chloride concentrations with no significant change for sodium concentration and an increase in creatinine level in male Wistar albino rats co-infected with malaria and *S. typhi*. The result in the other hand, contradicts the work of [35] who reported significant decrease in serum electrolytes (sodium, potassium and chloride) levels in patients with established typhoid infection, stating that it could be resulting from the electrolyte depletion due to diarrhoea and vomiting. Serum urea level was however in line with the result obtained in this study. As is the characteristic of dehydration commonly encountered in enteric fever, electrolyte derailment observed could be attributable to excessive water loss in faeces, and reduced water intake. In the treatment group, a significant ($p < 0.05$) dose dependent reduction was observed in serum urea, sodium, and chloride as well as significant ($p < 0.05$) increase in serum potassium and creatinine levels (Table 3). From these observations, one could infer that perhaps the extract and fraction were able to inhibit the growth of the *S. typhi*, enhancing rehydration via increased water intake, thus reducing water loss from stool and vomiting.

Liver injury can be accessed by the estimation of the concentrations of its enzymes in the blood, because the enzymes are confined to the liver and are found within a particular range in the blood. Any injury to the liver makes the enzymes seep out of the liver to the bloodstream. Activities of liver enzymes are related to the function of hepatocytes and therefore, an increase in the activity of alkaline phosphatase may be because of increased synthesis due to increased biliary pressure, while an increase in alanine and aspartate aminotransferases could be due to injury [36]. *S. typhi* infection led to a significant ($p<0.05$) increase in the activities of the liver enzymes (alkaline phosphatase, alanine and aspartate aminotransferase) as seen in Fig. 1 when compared with the positive control group. This may be a result of injury due to endotoxins released by the microorganism. Similar to this, is the study of [37] who reported that *S. typhi* provoked a significant increase in ALT, AST, ALP, albumin, bilirubin, creatinine, and uric acid levels compared to the positive control. Administration of the methanol extract and the ethyl-acetate fraction led to significant dose dependent reduction (Fig. 1) in these pa-

**Table 3**
Effect of *Ximenia americana* stem bark methanol extract and ethyl-acetate fraction on kidney function parameters of *Salmonella typhi* infected rats.

| Treatments | Sodium (mmol/L) | Potassium (mmol/L) | Chloride (mmol/L) | Urea (mmol/L) | Creatinine (mg/dL) |
|---|---|---|---|---|---|
| Positive Control | 73.33±11.70$^{a}$ | 1.90±0.03$^{e}$ | 80.67±7.31$^{a}$ | 13.33±0.88$^{a}$ | 0.83±0.15$^{c}$ |
| Negative Control | 87.50±2.50$^{c}$ | 0.59±0.10$^{a}$ | 88.50±10.50$^{d}$ | 21.00±8.00$^{d}$ | 0.60±0.10$^{a}$ |
| Standard Control | 75.33±3.18$^{ab}$ | 1.56±0.08$^{de}$ | 82.00±0.57$^{ab}$ | 13.53±0.26$^{ab}$ | 0.81±1.83$^{c}$ |
| ME 100 | 84.33±3.38$^{c}$ | 0.83±0.15$^{ab}$ | 85.33±2.91$^{bc}$ | 17.67±1.45$^{c}$ | 0.70±0.03$^{b}$ |
| ME 200 | 77.00±0.58$^{ab}$ | 1.00±0.32$^{c}$ | 84.00±3.21$^{b}$ | 14.83±0.17$^{b}$ | 0.80±0.06$^{c}$ |
| ME 300 | 74.00±3.79$^{ab}$ | 1.38±0.29$^{cd}$ | 83.33±1.86$^{b}$ | 13.33±1.45$^{a}$ | 0.88±0.03$^{c}$ |
| EF 100 | 80.00±9.17$^{ab}$ | 0.70±0.32$^{b}$ | 86.00±3.78$^{b}$ | 19.03±3.75$^{b}$ | 0.66±0.08$^{ab}$ |
| EF 200 | 78.00±12.29$^{a}$ | 0.93±0.33$^{c}$ | 84.40±28.28$^{ab}$ | 17.33±8.09$^{b}$ | 0.73±0.09b$^{c}$ |
| EF 300 | 77.00±1.53$^{a}$ | 1.20±0.21$^{d}$ | 82.33±0.88$^{a}$ | 14.33±2.33$^{a}$ | 0.83±0.88$^{c}$ |

Values are mean ± standard error of three replicates ($n = 3$). Values with different alphabets along a column are significantly different ($p < 0.05$).
KEY: ME- Methanol extract; EF- Ethyl-acetate fraction (Doses are in mg/kg body weight).

rameters specifically for ALP. These observations could be as a result of the extracts been able to prevent the excessive synthesis of the enzymes, inhibit their release from hepatocytes and therefore, ameliorating the injury.

The significant increase in the total and conjugated bilirubin levels observed in the *S. typhi* infected group when compared to the positive control group (Fig. 2) is in line with previous studies of [36]. It can be inferred to endogenous production of oxidizing substance which is responsible for auto-oxidation in addition to increased haemolysis. Treatment with both extracts led to a dose dependent decrease (Fig. 2) in total and conjugated bilirubin levels.

There was a significant ($p < 0.05$) as well as a non-significant ($p > 0.05$) decrease in total protein and albumin levels in the negative control group when compared to the positive control (Fig. 3). The result is similar to that of [38] who evaluated the serum liver enzyme markers, lipid profile and kidney function parameters in human typhoid patients, relating the result to kidney malfunction. [39] had a similar result for total protein in their work and attributed it to enteric protein loss due to changes in the integrity of the intestinal mucosa. In the same way, we relate our findings of decreased total protein levels to its reduced absorption following the disruption of integrity of the stomach lining. Groups of rats that received treatment had their total protein and albumin concentrations that compared favourably to that of the positive control group (Fig. 3).

Bacterial infections amongst other factors have been shown to have an inimical effect on the haematological indices of the host organism involved in the infection. In living cells, these infections prompt cell damage when they exist in the host organisms by releasing toxins that alter certain processes in the host metabolism and consequently leading to a rise in free radical species [32]. In this study, *S. typhi* infection significantly ($p < 0.05$) resulted in decreased haemoglobin concentration (Fig. 3), mean cell haemoglobin concentration (Fig. 3) red blood cell (RBC) count (Fig. 4), mean cell haemoglobin (MCH) (Fig. 5), and packed cell volume (PCV) (Fig. 7) as well as increased mean cell volume (MCV) (Fig. 6) as compared to the positive control. The observation agrees with the works of [32,33]. The effect of *S. typhi* infection according to the authors may be because of the interaction between the bacteria toxins and the blood-forming tissues which in return inhibits the rate at which stem cells possessing hematopoietic specializations are synthesized. In addition to this, [40] added that these toxins can interact with the red blood cell membrane proteins to accelerate the rate of red cell destruction. Thus, from the above, it can be inferred that the decrease observed in red blood cell count, haemoglobin concentration, and packed cell volume in might be due to retardation of hemopoiesis and increased haemolysis of red cells. Both the methanol extract and the ethyl-acetate fraction of *X. americana* stem bark elicited significant ($p < 0.05$) increase in PCV and haemoglobin concentration in a dose dependent manner as shown in Figs. 3 and 7 with the values obtained having no significant ($p > 0.05$) difference when compared with the positive control. This could be attributed to the ability of the extracts to reverse the damages prompted by the presence of the bacteria in the bloodstream viz; improving hemopoietic activity of specialized stem cells and restoring membrane integrity [41].

The significant ($p < 0.05$) increase in lymphocyte, total white blood cell count, monocyte, eosinophil, basophil (M,E,B) and decrease in neutrophil observed in the group infected with *S. typhi* when compared with the normal control group (Figs. 7 and 8) was consistent with the work of [42] but the total white blood cell count decreased in his study which is contrary to this present study. According to [42], a significant increase in lymphocytes, eosinophils, and monocytes in the infected group may likely be a result of the allergic response to the infection. [43] in a different study however, reported an increase in total white blood cell count in *S. typhi* infection that was attributed to the enormous production of white cells in response to the infection. Thus, the resultant decrease in total white blood cell count, lymphocyte, and increase in neutrophils in the treatment groups (Figs. 7 and 8) could be as a result of inhibition of the growth of *S. typhi* by the extract and fraction leading to the destruction of these cells and reduction of phagocytosis.

## 5. Conclusion

Studies on the methanol extract and its ethyl-acetate fraction of *X. americana* revealed that it contains secondary metabolites that tend to be responsible for the observed antimicrobial activity, buttressing the claim for its use in folkloric medicine in the treatment of enteric infections. More so, histopathological studies on hepatocytes and nephrocytes are required to confirm the safety of the extracts. The study has however provided evidence on this direction.

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## Declaration of Competing Interest

The authors declare no conflict of interest.

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