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Therapeutic Effect of Vernonia Amygdalina Aqueous Leaf Extract on Salmonella Typhimurium-Infected Female Wistar Rats

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ABSTRACT

This study investigated the therapeutic effects of Vernonia amygdalina aqueous leaf extract administration on Salmonella typhimurium infected Wistar rats. Rats were infected orally by a single dose administration of Salmonella typhimurium (1.5 x 10^8 CFU). Negative control groups were infected and treated orally with vehicle (distilled water), neutral control group were not infected, while the four test groups were treated up to 24 days with 50 mg/kg. 100 mg/kg, 150 mg/kg and 200 mg/kg body weight of aqueous leaf extract of V. amygdalina respectively. The effects of leaf extract administration on serum markers (amino transferase activities, total protein, creatinine and bilirubin levels), as well as histopathology of the liver and kidney tissues were also investigated. Following in vivo studies, the 50 mg/kg dose of aqueous leaf extract of V. amygdalina was effective in alleviating liver damage as seen in the amino transferase activity. Infection resulted in a significant increase of amino transferase activity. The drug and plant extract helped to alleviate liver and kidney damage from infection as observed in the organ weights and their protein content. Findings from this study showed that the administration of this aqueous leaf extract at higher doses resulted in the ameriolation of the tissue damage caused by the S. Typhi bacteria (from histological studies). These results support the ethnomedicinal use of V. amygdalina, and posits that its leaves can be used in antibacterial phytomedicine.

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INTRODUCTION

Typhoid fever, also known as enteric fever is an acute illness caused primarily by the *Salmonella enterica* serotype *Typhi* bacteria, and to a lesser extent, by the *S enterica* serotypes *paratyphi* A, B, and C [1, 2]. It is usually spread through food or water contaminated with infected animal or human feces. Worldwide, an estimated 26 million cases of typhoid fever occur annually, causing 215,000 deaths, with a vast majority of infections and deaths taking place in developing countries [3].

Symptoms associated with *Salmonella* infection include fever, severe headaches, abdominal pain, constipation, diarrhoea, vomiting, loss of appetite, rose spot, anorexia and hepato-splenomegaly [4]. These symptoms may be mild or severe and usually begin six to thirty days after exposure [5].

Although the production of new antibiotics by pharmacological industries has taken place in the last three decades, drug-resistance by microorganisms to these agents has increased, and hence, the continued search for alternative drugs [6]. In addition, some of these drugs have detrimental side effects and can be hurtful to individuals or patients. An example is the drug, chloramphenicol. Apart from pharmacological treatments, various therapeutic approaches have been used since time immemorial for many health ailments [7]. This includes plant-based traditional medicine, also known as herbal medicine, and many modern drugs have been isolated from natural (or plant) sources.

Some herbs are known for their medicinal potentials and effects. Herbal medicine is the use of plants to treat disease and enhance general health and wellbeing. It has played and continues to play an important function in the healthcare of many populations. According to the World

Health Organization (WHO), approximately eighty percent of the World population still depends upon the use of herbal remedies for their health care [8]. In developing countries, the use of plant extracts is prompted by the increasing poverty of people who cannot afford modern drugs.

Vernonia amygdalina Del., commonly known as bitter leaf, is a shrub or small tree that can reach up to 6-7 metres in height when fully grown. It belongs to the Asteraceae family, a native to Africa and grows in most parts of Sub-Saharan Africa [9]. It is reported to have potent medicinal properties, such as antidiabetic, antihypertensive, anticancer, antiviral, and immunomodulatory activities [10] [11]. A number of scientific works have been done, and they report the antibacterial or antimicrobial activity of *V. amygdalina* [12] [13]. In West African regions, leaves of *V. amygdalina* are used for the treatment of diarrhoea, malaria, fever, intestinal complaints, and for the management of diabetes [12, 13]. Considering the traditional uses and previous research carried out *in vitro* on this plant, the present study intended to investigate the *in vivo* antibacterial activity of its aqueous leaf extract on *Salmonella typhimurium*-infected rats.

This study aims at validating using scientific information on the use of *V. amygdalina* Del., as an antibacterial activity and efficacy in the treatment of typhoid fever.

MATERIALS AND METHODS

Plant and animal materials

Fresh *Vernonia amygdalina* (bitter leaves) were harvested from a farm in Ipaja area in Lagos State, Nigeria. Two kilograms of the *V. amygdalina* fresh leaves were washed and dried at room temperature. Fifty albino male Wistar rats were purchased from the laboratory animal centre of the College of Medicine, University of Lagos, Lagos. The laboratory animals and the fresh leaves were used for the study. Ethical approval was obtained from the health ethics research committee of the College of Medicine, University of Lagos, Lagos (HREC 021/00227). They were treated in accordance to OECD2008a/407 [14] guidelines for Repeated Dose Oral Toxicity Study in Rodents.

Bacteria species

Salmonella typhimurium isolate was obtained from the Lagos University Teaching Hospital (LUTH) Lab (Idi-Araba, Lagos).

Bacteria culture media and preparation of inocula

In this study, two culture media were used, namely, MacConkey agar and Nutrient agar. The bacterial cell suspensions were prepared at $1.5x10^8$ colony-forming units/ml (CFU/ml) following N° 0.5 McFarland turbidity standard. 20h old overnight bacterial cultures were prepared on MacConkey agar and Nutrient agar, and few bacterial colonies were transferred aseptically with a sterile loop into 10ml of sterile 0.90% saline distilled water and homogenised.

Preparation of the plant extract

V. amygdalina leaves were picked, washed thoroughly and macerated. After this, the leaves were put in a blender. Water was added to the blender and the content was left to spin. Next, the slurry was transferred to a sterile container. The process was repeated until all the leaves were ground. The slurry in the sterile container was sieved using a fine sieve. The residue were discarded leaving the *Vernonia amygdalina* aqueous leaf extract. The plant extract was transferred to a sterile container and was kept frozen until further use.

Preparation of the required doses of plant extract

A certain volume of the prepared stock solution of *Vernonia amygdalina* leaf extract was poured out into a metal pan which had been weighed previously. The metal pan containing the aqueous leaf extract was then placed in a hot air oven. After the concentration of the solution, the metal pan containing the dried extract was weighed, and the weight and volume of the concentrated stock solution was then determined. These values were used to determine the actual concentration of the prepared stock solution.

Using the $C_1V_1=C_2V_2$ formula, the required doses (50 mg/kg, 100 mg/kg, 150 mg/kg, and 200 mg/kg) of *V. amygdalina* leaf extract were prepared from the stock solution using dilution method. They were kept in labelled plastic bottles and stored in a refrigerator at -4˚C when not in use.

Animal treatment

For this study, 28 female Wistar rats aged between 5 and 7 weeks were used. Prior to their utilization, animals were immunosuppressed by oral administration of 30 mg/kg bw of cyclophosphamide for three consecutive days as previously described by Abhishek *et al.* [15].

Animals were arranged into seven groups of four animals each according to their average body weight. With the exception of group 1 animals, they were infected by the oral administration of 1 ml of a suspension containing 1.5×10^8 CFU of *S. typhimurium* prepared at 0.5 Mc Farland turbidity scale.

Animal Treatment and Handling

The animals were treated as follows:

Group one (neutral control) was not infected and received distilled water during the treatment period.

Group two (negative control group) was infected, but not subsequently treated and animals in this group received only distilled water during the treatment period.

Group three (positive control) received 5 mg/kg body weight of oxytetracyclin [16] during the treatment.

Animals of other groups (four to seven) were treated from the seventh day after infection, with different doses of the plant extract (50, 100, 150 and 200 mg/kg body weight).

During 24 days of treatment, animals were given the treatment before allowing them to feed and drink water *ad libitum*. Animals were weighed daily and their body temperature was also recorded. Temperature records were

taken every two days following the day after infection using a thermometer through the anal orifice.

Evaluation of biochemical parameters

On the 16th day of treatment, blood samples were obtained from two rats of each Group from 1 to 7. Blood was collected from the retro-orbital sinus of the rats using capillary tubes and fed into sterilized blood tubes. The blood samples were collected into lithium heparin tubes. It was then spun in a centrifuge at 2000 rpm for 10 min. The plasma obtained were used for the determination of biochemical parameters.

On the 24th day of treatment, blood samples were obtained from three rats of each group as described earlier. They were also collected into lithium heparin tubes and the plasma separated was used for determination of biochemical parameters. After blood collection, animals were sacrificed and their organs, such as heart, liver, spleen, kidneys and lungs were excised, weighed and their protein contents determined.

Biochemical analysis

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, lipid profile [total cholesterol, high density lipoprotein (HDL) and triglyceride levels], bilirubin, creatinine and urea levels were determined using Roche Hitachi cobas c311 clinical chemistry analyzer. The low density lipoprotein (LDL) was calculated using the formula of Friedewald et al. [17]: LDL = TC - HDL- (TG/5); while atherosclerosis index (LDL/HDL) was calculated using Mertz's formula [18]. Total tissues (liver and kidneys) protein levels were measured by the Lowry method.

Preparation of organ homogenates

The animals were sacrificed on the 24th day of treatment. The organs of the rats (the heart, liver, spleen, kidneys and lungs) were harvested and weighed using a sensitive weighing balance. The liver and kidney of individual rats from each group were ground in 4 ml of phosphate buffer. The resultant mixture was centrifuged at 3,000 rpm for 5 min and the supernatant was taken and preserved at -15˚C. The supernatant obtained was used for determination of total tissues protein using Lowry assay.

Histological study

The liver and kidneys from individual rats were harvested following rat sacrifice and fixed immediately in 10% neutral buffered formalin, which was used for histological study. Histological slides were photomicrographed using a photomicroscope. The reading was done using a light microscope. Histopathology was determined based on severity of changes compared to control sections.

Statistical analysis

Data obtained were expressed as mean±SEM (standard error of mean) and were analysed using One way ANOVA. Tukey's test was used for post hoc analysis. A probability value less than 0.05 was considered statistically significant.

RESULTS

In vivo therapeutic properties

Table 1 shows that on the 16th day the activities of circulatory AST were significantly higher in animals of the neutral control as well as the 100mg/kg group. There was a significant decrease of AST activity in infected animals treated with 50mg/kg as compared to negative and neutral control. Animals of the other treated groups showed no decrease as compared to negative control. On the other hand, the activities of ALT were not significantly high in the neutral control group. There was a decrease of ALT activity in infected animals treated with 50mg/kg as compared to neutral control. There was a decrease in the 100mg/kg group compared to negative control.

Comparing the AST activities of the 16th and 24th days, it was observed that the animals of the 200mg/kg group showed the most significant reduction in AST activities over time. This was followed by the neutral control, 50mg/kg and 150 mg/kg groups respectively. However, the 100 mg/kg group showed a remarkably low reduction, followed by the negative control group.

Table 1. Effect of administration of aqueous leaf extract of *Vernonia amygdalina* on AST and ALP activities of rats on the 16th and 24th days of treatment.

Group	Period (day)	AST (IU/L)	ALT (IU/L)
Ui/Ut	16	189.70±1.00	38.75±0.65
	24	121.00±20.30	37.85±2.85
I/Ut	16	166.80±18.00	41.75±4.65
	24	163.75±11.45	35.85±1.85
Oxytet.	16	183.60±22.40	41.10±3.40
	24	134.60±21.50	36.55±2.75

50 mg/kg	16	148.00±13.60	37.15±4.05
	24	92.40±8.50	38.35±5.45
100 mg/kg	16	207.00±6.20	40.95±4.45
	24	198.35±18.75	61.90±10.0
150 mg/kg	16	172.30±11.40	42.00±2.80
	24	137.90±33.40	27.95±3.45
200 mg/kg	16	175.30±0.40	44.40±12.20
	24	105.30±0.30	29.25±5.25

Ui/Ut: Uninfected and untreated; I/Ut: Infected and untreated; Oxytet.: Group treated with drug (oxytetracycline). Values are mean±SEM of two trials.

Table 2. Effect of administration of aqueous leaf extract of *Vernonia amygdalina* on bilirubin, creatinine and blood urea levels of rats on the 16th and 24th days of treatment

Group	Period (day)	Bilirubin (µmol/L)	Creatinine (µmol/L)	Urea (mmol/L)
Ui/Ut	16	2.35±0.25	67.00±4.30	8.30±0.00
	24	1.50±0.40	41.85±0.65	5.35±0.05
I/Ut	16	2.30±0.20	71.40±12.30	8.00±0.60
	24	1.35±0.45	39.95±5.95	11.35±3.25
Oxytet.	16	2.50±0.20	57.80±2.10	7.45±0.65
	24	1.40±0.10	37.60±0.30	6.25±0.15
50 mg/kg	16	2.15±0.15	57.30±4.40	7.60±0.40
	24	1.75±0.32	37.85±5.25	6.25±0.75
100 mg/kg	16	1.80±0.80	66.00±4.20	7.85±0.65
	24	1.50±0.30	49.30±0.60	13.20±5.10
150 mg/kg	16	2.30±0.30	68.80±10.60	8.75±1.25
	24	1.15±0.27	41.60±0.30	6.20±0.20
200 mg/kg	16	1.95±0.25	48.85±3.35	6.55±0.75
	24	1.10±0.07	43.20±2.60	6.25±1.65

Ui/Ut: Uninfected and untreated; I/Ut: Infected and untreated; Oxytet.: Group treated with drug (oxytetracycline). Values are mean±SEM of two trials.

From the values obtained on the 16th day, it was observed that infection did not result in a significant increase of bilirubin levels (Table 2). The infected and treated animals of the 100mg/kg group showed lowest levels of bilirubin, followed by the 200mg/kg group. The infection of animals resulted in an increase in creatinine levels followed by a significant decrease in 50mg/kg and 200mg/kg groups as compared to neutral control. Except for the 150mg/kg group, all groups treated with extract had decreased levels of urea as compared to both negative and neutral control, with the 200mg/kg group being the most significant.

Comparing the values of the 16th and 24th days of treatment, it was observed that the animals that received the 150 mg/kg doses of the extract showed the most significant

reduction in bilirubin levels, followed by the 200 mg/kg, 50 mg/kg and 100 mg/kg groups respectively. The negative control group also showed a significant reduction in bilirubin levels as well. As for the values of plasma creatinine obtained, the negative control group showed the most significant decrease over time. Among the treated groups, the 150 mg/kg group had the most significant reduction in creatinine levels, followed by the 50 mg/kg, 100 mg/kg and 200mg/kg groups respectively. The negative control group and the 100 mg/kg group showed an increase in blood urea levels over time. However, the neutral control group had the most significant decrease in urea levels. Among the treated groups, reductions were observed among the 150 mg/kg, 50 mg/kg and 200 mg/kg groups respectively.

Table 3 shows that on the 16th day the infection resulted in an increase of total cholesterol levels. The group receiving the 150 mg/kg dose of the extract showed a significant decrease as compared to negative and neutral control. The 50 mg/kg group also showed a small decrease as compared to negative control. Concerning HDL levels, the 50 mg/kg and 100 mg/kg groups showed higher values as compared to negative control. However, only the LDL levels of the 100 mg/kg and 200 mg/kg groups were increased as compared to negative control.

Comparing the values of the 16th and 24th days of treatment, it was observed that the animals of the 150 mg/kg group showed the most significant increase of HDL levels over time. The neutral control also showed a significant increase in HDL levels. Among the other treated groups, the 50 mg/kg group also showed a small increase in HDL levels. The 100 mg/kg and 200 mg/kg groups decreased in HDL levels, respectively. The LDL levels of the animals increased in the neutral and negative control groups, as well as the 50 mg/kg group. There was a decrease of LDL in the other treated groups.

Table 3. Effect of administration of aqueous leaf extract of *Vernonia amygdalina* on total cholesterol, HDL, and LDL levels of rats on the 16th and 24th days of treatment.

Group	Period (day)	Total cholesterol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Ui/Ut	16	1.86±0.15	0.89±0.12	0.87±0.02
	24	2.68±0.43	1.10±0.07	1.37±0.46
I/Ut	16	2.07±0.17	1.12±0.12	0.88±0.05
	24	1.99±0.53	0.80±0.08	1.03±0.56
Oxytet.	16	1.77±0.01	0.86±0.01	0.83±0.10
	24	1.96±0.31	0.93±0.03	0.89±0.31
50 mg/kg	16	2.05±0.02	1.13±0.12	0.82±0.12
	24	2.33±0.35	1.15±0.16	1.32±0.75
100 mg/kg	16	2.25±0.47	1.24±0.31	0.93±0.17
	24	1.61±0.43	0.79±0.13	0.68±0.26
150 mg/kg	16	1.63±0.02	0.71±0.01	0.86±0.00
	24	2.17±0.24	1.19±0.24	0.80±0.19
200 mg/kg	16	2.10±0.18	1.08±0.23	0.95±0.07
	24	1.77±0.40	1.05±0.15	0.85±0.34

Ui/Ut: Uninfected and untreated; I/Ut: Infected and untreated; Oxytet.: Group treated with drug (oxytetracycline). Values are mean±SEM of two trials.

Table 4. Effect of administration of aqueous leaf extract of *Vernonia amygdalina* on triglyceride level and atherogenic index of rats on the 16th and 24th days of treatment.

Group	Period (day)	Triglycerides (mmol/L)	Atherogenic index
Ui/Ut	16	0.47±0.02	0.99±0.11
	24	1.07±0.07	0.85±0.38
I/Ut	16	0.40±0.04	0.79±0.04
	24	0.79±0.26	0.56±0.07
Oxytet.	16	0.43±0.01	0.96±0.02
	24	0.71±0.06	1.08±0.62
50 mg/kg	16	0.51±0.07	0.74±0.18
	24	0.84±0.17	0.48±0.16
100 mg/kg	16	0.46±0.00	0.77±0.06
	24	0.69±0.14	0.83±0.19
150 mg/kg	16	0.26±0.03	1.21±0.02
	24	0.87±0.10	0.49±0.13
200 mg/kg	16	0.38±0.07	0.94±0.26
	24	1.13±0.29	0.45±0.01

Table 4 shos that on 16th day, the 150 mg/kg and 200 mg/kg groups showed decreased levels of triglycerides compared with negative and neutral control. However, the other treated groups (50 mg/kg and 100 mg/kg) showed increased levels compared to negative control. As far as atherogenic index is concerned, the 50 mg/kg and 100 mg/kg groups showed decreased levels compared to the negative control.

Comparing the values of the 16th and 24th days of treatment, it was observed that animals of the 150 mg/kg and 200 mg/kg showed the most significant increase in triglyceride levels over time. The 50 mg/kg and 100 mg/kg groups also increased in triglyceride levels, but had lower increments compared to negative and neutral control. The animals of the 150 mg/kg and 200 mg/kg groups showed the most significant reductions in atherogenic index over time, and also recorded the lowest values among all the groups. This was followed by the 50 mg/kg group and neutral control group which also decreased in atherogenic index values over time. However, the 100 mg/kg group showed an increase in values over time.

Effect of *V. amygdalina* aqueous extract on hepatic and renal tissues' protein rate of rats

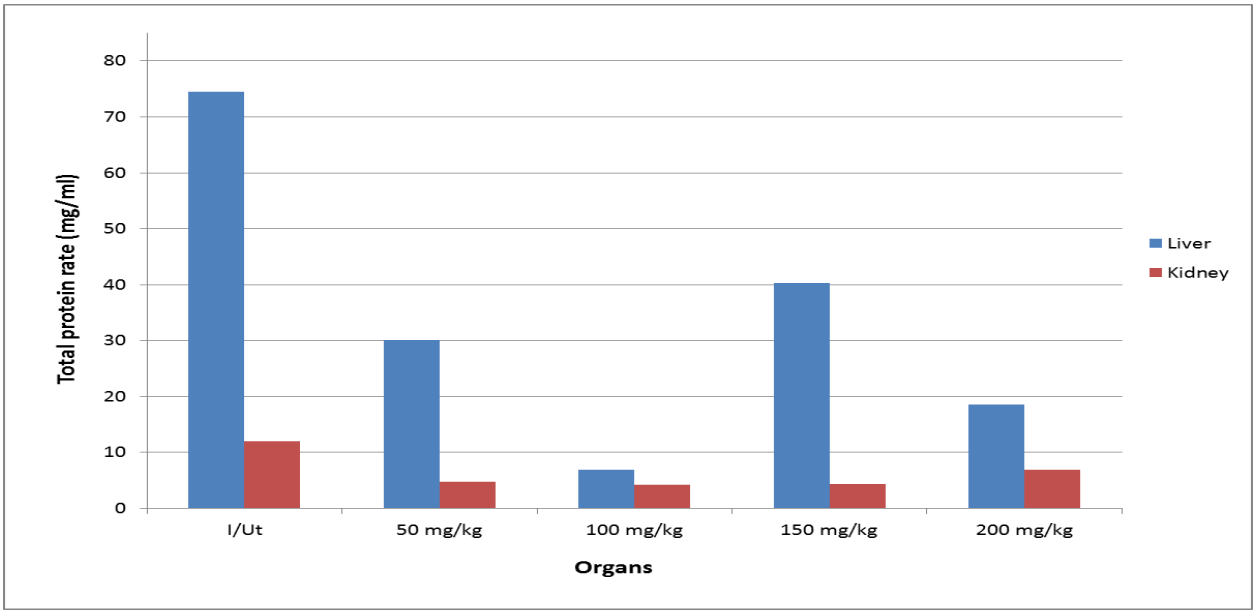


Fig. 1. Total protein rates of whole rat liver and kidneys as a function of dose in tested animals. I/Ut: Negative control. Values are of individual (single trials).

The protein rate in tissues of the liver and kidneys of the animals was assessed at the end of the treatment and the results is presented in Fig. 1. The hepatic protein rate of the negative control was significantly high and decreased in a dose-dependent manner in animals treated at 50 mg/kg and 100 mg/kg. However, the 150 mg/kg group recorded an increase in protein rate compared to the other treated groups. The renal protein rate of the negative control was also relatively high compared to the other treated groups. However, the values decreased across the 50 mg/kg and 100 mg/kg groups, with a slight increase recorded again across the 150 mg/kg and 200 mg/kg groups.

Effect of *V. amygdalina* aqueous leaf extract on relative organ weights of rats

The effect of treatment on the relative weight of rat organs such as the heart, liver, spleen, kidneys and lungs is shown in Fig. 2.

It was observed that treatment with different doses of extract resulted in some differences of relative organ weights among the treated groups compared to controls, especially in the 50 mg/kg and 200 mg/kg groups. There was a significant increase of spleen relative weight at 100 mg/kg, after which a dose-dependent decrease was observed at 150 mg/kg and 200 mg/kg. The kidney relative weight increased dose-dependently across all the treated groups. There was no increase of liver relative weight upon infection, but such were observed amongst the 50 mg/kg, 150 mg/kg and 200 mg/kg groups.

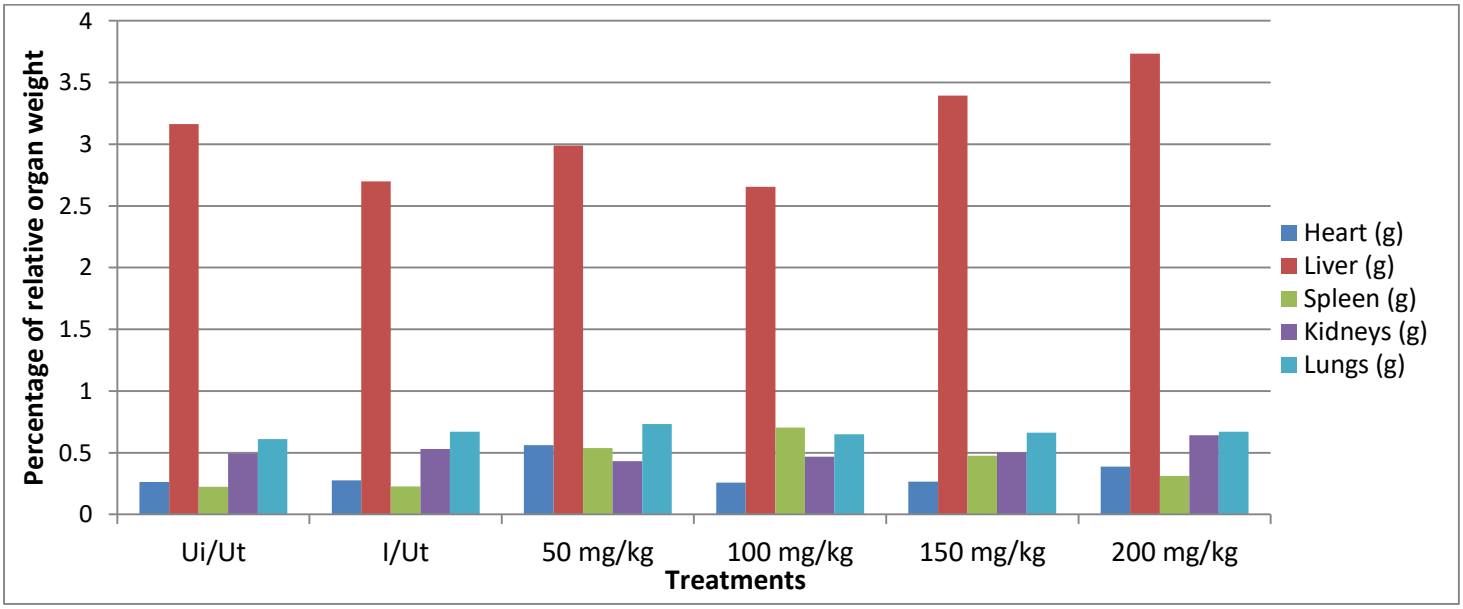


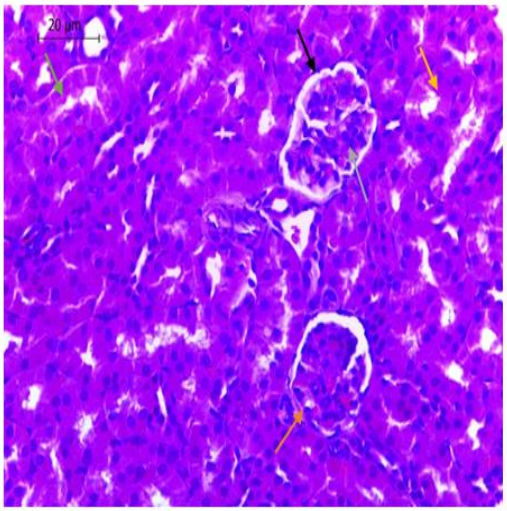
Fig. 2. Percentage of relative organ weight. Ui/Ut: Neutral control, I/Ut: Negative control. Values are of individual (single) trials.

Histological profiles of hepatic and renal cells

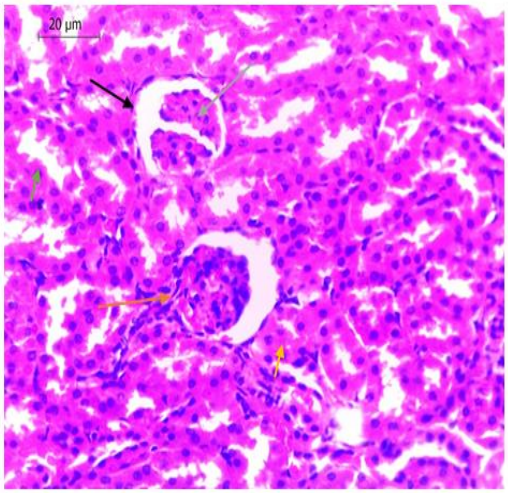
The photomicrographs of rat liver and kidney tissues from each group are shown in Fig. 3. The photomicrographs of the kidneys are shown first, followed by the liver.

KIDNEY SECTIONS

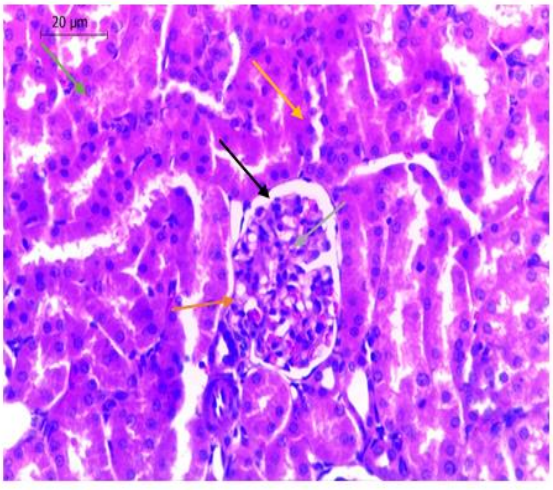
Ui/Ut (Kidney)



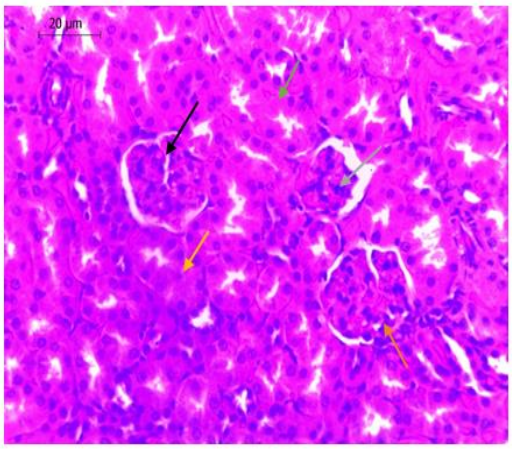
I/Ut (Kidney)



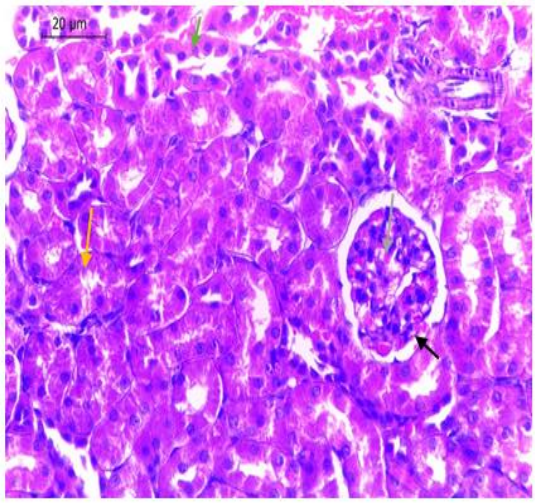
Group 3 (Kidney)



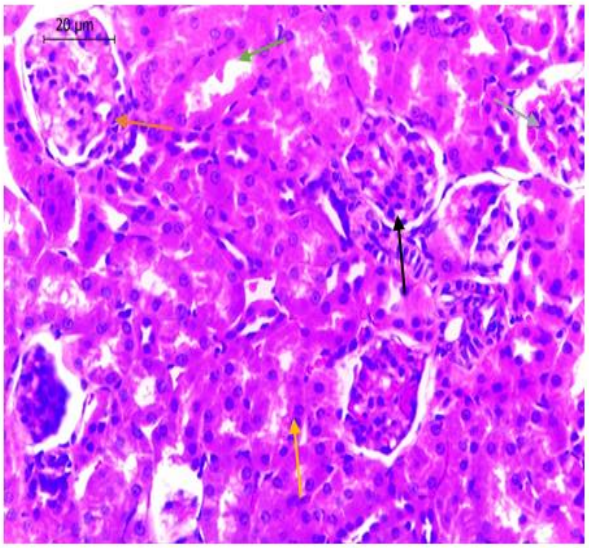
Group 4 (Kidney)



Group 5 (Kidney)



Group 6 (Kidney)



Group 7 (Kidney)

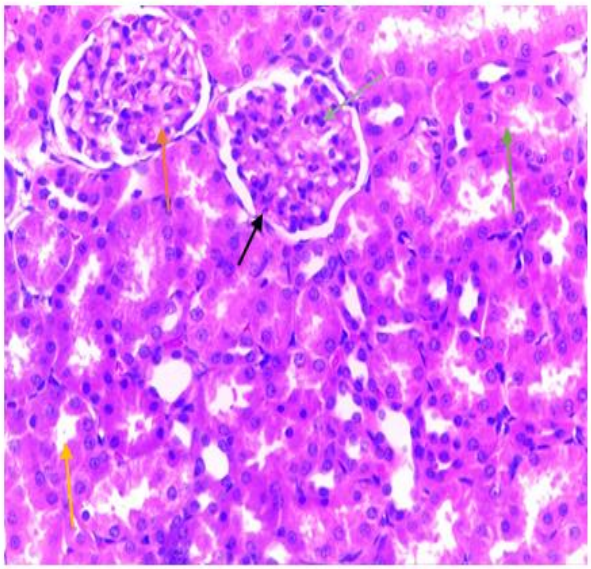


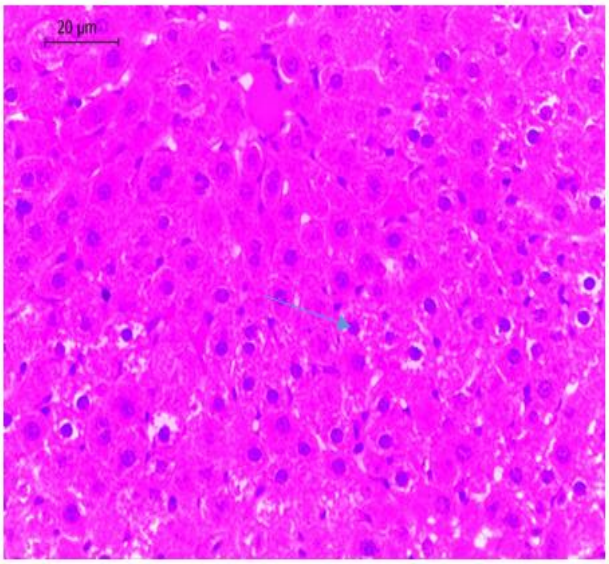
Fig. 3: Liver photomicrographs of Samonella infected rats administered aqueous leaf extract 0f *V. amydallina*

Photomicrograph of kidney (H& E stain) showing normal dark staining of the renal cortex that containing normal cellular glomerular tufts in black arrow, proximal convoluted tubules in yellow arrow, distal convoluted tubules in green arrow as well as light staining renal medulla. The architecture of the glomerulus is intact with a well distinct vascular pole in red arrow and dispersed podocyte in ash arrow. No form of abnormalities is found.

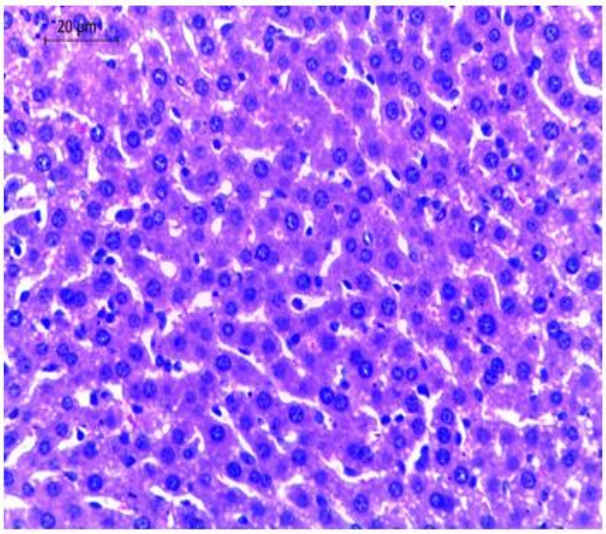
COMMENT: ALL ARE NORMAL WITHOUT ANY ABNORMALITY

LIVER SECTIONS

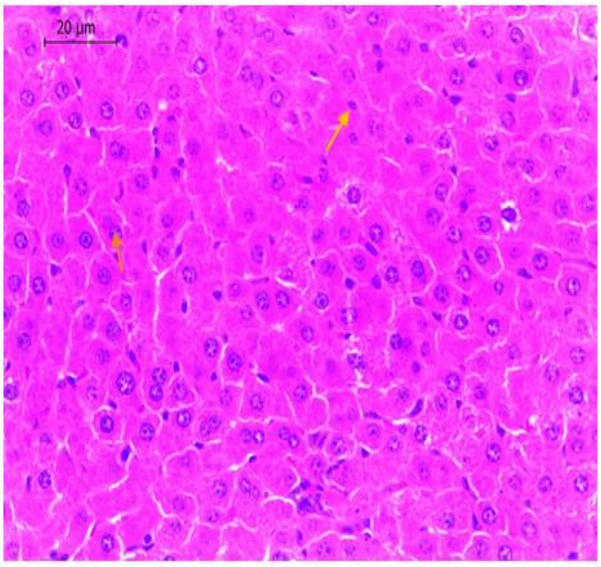
Ui/Ut



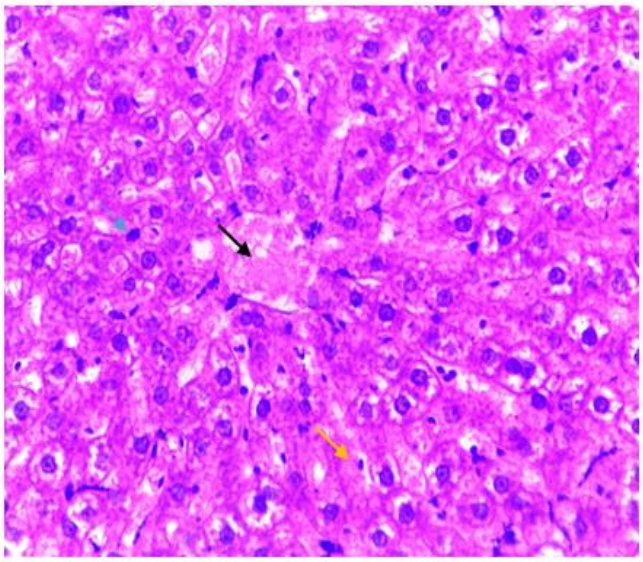
I/Ut



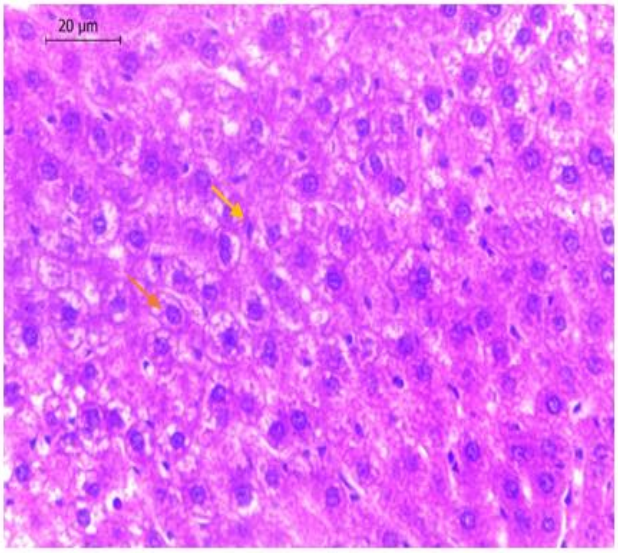
Group 3 (Liver)



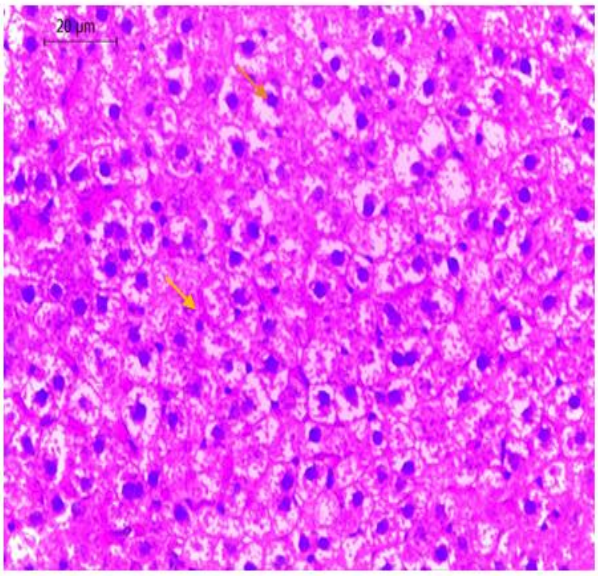
Group 4 (Liver)



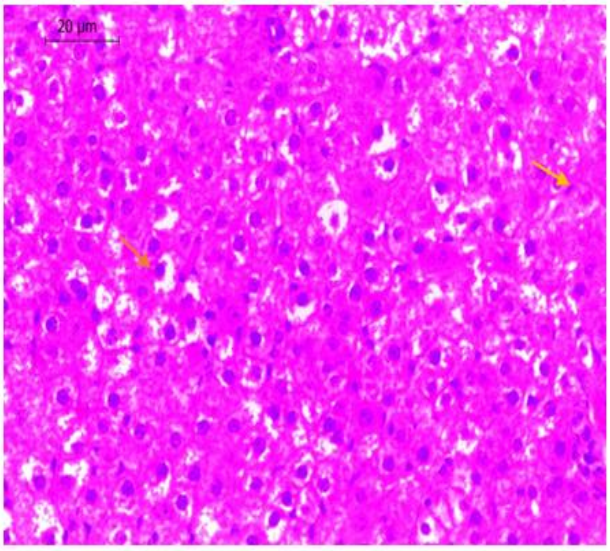
Group 5 (Liver)



Group 6 (Liver)



Group 7 (Liver)



Photomicrograph of liver (H & E stain) showing normal central vein with a normal plate of hepatic cell (hepatocyte) in red arrow that radiate parallel from the Central vein (CV) in black arrow. The architecture of the portal vein (PV) in green arrow, the sinusoid and the endothelial cell lining the sinusoid is normal, in yellow arrow. No form of abnormalities is found.

GENERAL COMMENT: ALL ARE NORMAL WITHOUT ANY ABNORMALITY.

DISCUSSION

The onset of infection with *S. typhimurium* was observed as there were some changes in the physiology of the animals, such as excretion of watery stool, the presence of mucus in the stool, a decline in activity, and constipation in some of the rats a few days after administration of infectious load. Some days after treatment had commenced, many of these symptoms were alleviated and there was a general improvement in the health of most of the rats. This may be due to the combined action of the extract and the immune system of the rats. The results suggest that the extract has some therapeutic effect in *S. typhimurium* infection and has potential for the treatment of typhoid fever disease.

The AST activities on the 16th day were elevated in most of the groups, including the uninfected and non-treated group. This may be due to other factors causing liver damage in the rats. However, these levels reduced substantially from the 16th to the 24th day among the treated groups (except the 100 mg/kg group), with the 200 mg/kg group showing the most significant reduction. This may be due to liver injury of animals due to infection which might still been present on the 16th day but was being corrected up till the 24th day of extract administration. Only the 50 mg/kg and 100 mg/kg treated groups showed lower activities of ALT compared to the negative control group on the 16th day. However, the 150 mg/kg and 200 mg/kg groups showed a substantial decrease in ALT activities from the 16th to the 24th day. This suggests that the extract at these doses would correct liver cell damage and induce a hepatoprotective effect. This result is in agreement with the findings of Arhoghro *et al*. (2009) who found that *V. amygdalina* leaf extract alleviates chemically-induced liver damage in albino Wistar rats. This was also corroborated by the relative organ weight results which show dose-dependent increases in liver weights across the negative control and treated groups (except the 100 mg/kg group). However, substantial decreases were observed in liver and kidney protein contents among the negative control and treated groups, which does not support this fact (hepatoprotective effect). Due to the injury of these tissues, the protein content could have moved into the blood circulation. This result obtained may also be due to the lack of generalizability due to low sample size (single trial) used for protein content determination.

The values obtained for the 100 mg/kg group are somewhat unusual when compared to the other treated groups, and this

may be due to the fact that the animals of the group may have had other underlying conditions which made them very ill. It may also be due to low immunity of the rats in this group and the negative effect of the immunosuppressant (cyclophosphamide) used on them.

Infection did not result in a significant increase in bilirubin levels as observed on the 16th day. However, decreases were observed among some of the treated groups such as the 50 mg/kg, 100 mg/kg and 200 mg/kg groups. From the 16th to the 24th day, the most significant reduction was observed among the 150 mg/kg group, followed by the 200 mg/kg group. This suggests that the damage to the liver caused by infection with *S. typhimurium* bacteria may not have been very severe but was further healed by the extract up till the 24th day of treatment.

The high plasma creatinine levels observed in the infected and non-treated group on the 16th day (negative control) indicates kidney damage. This was followed by significant decreases among some of the treated groups (50 mg/kg and 200 mg/kg) which suggest that the extract might have a kidneyprotective effect. Furthermore, there were substantial reductions in creatinine levels from the 16th to the 24th day among some of the treated groups. The values obtained for blood urea nitrogen (BUN) are somewhat similar except for the 100 mg/kg group which had unusual results when compared to the other treated groups. This suggests that infection might have caused damage to the kidneys leading to a decrease in the elimination of creatinine and urea in the urine, but the extract could have corrected this kidney damage in some of the treated animals as evidenced by the results.

From the values obtained on the 16th day, the administration of extract did not result in significant changes of total cholesterol in the rats, except for the 150 mg/kg treated group. This is also true for the HDL levels, except for the 150 mg/kg group which showed a significant decrease in HDL levels. The levels of LDL were increased and decreased in some groups compared to negative control. There was a dose-dependent decrease in triglyceride levels among the 50 mg/kg, 100 mg/kg and 150 mg/kg treated groups on the 16th day. The values for the atherogenic index showed reductions from the 16th to the 24th day among most of the treated groups, especially the 150 mg/kg and 200 mg/kg groups. This indicates that the extract may be effective in preventing cardiovascular disease at these doses, and in attenuating triglyceride levels. These results are in agreement with the findings of earlier studies which report the lipid-lowering and anti-atherogenic effects of *V. amygdalina* extract (Adaramoye *et al.*, 2008; Omede *et al*., 2018).

Histological sections reveal some changes in the structure of the tissue sections, however, no striking difference was observed among the groups. There were all generally normal with no abnormality. This indicates that on the 24th day of treatment, damage to the liver and kidneys were corrected by both the action of the immune system and the extract used.

CONCLUSION

This study has found that the *Vernonia amygdalina* aqueous leaf extract is effective in alleviating liver damage caused by *Salmonella* bacteria, as seen in the amino transferases (AST & ALT) activities obtained. The study also showed that the *Vernonia amygdalina* aqueous leaf extract is effective in alleviating damage to the kidneys caused by *Salmonella typhimurium*, as seen in the creatinine values obtained. This study has found that the *Vernonia amygdalina* aqueous leaf extract was effective in increasing liver protein content among the test group suggesting its hepatoprotective effect. The study also found that the *Vernonia amygdalina* aqueous leaf extract was effective in increasing kidney protein content among the test group suggesting its kidneyprotective effect. This study found that the *Vernonia amygdalina* aqueous leaf extract was effective in increasing relative organ weights of the heart, liver, kidneys and lungs of rats. This finding reports its organ protective effect. The histopathology studies revealed that the 200mg/kg dose of *Vernonia amygdalina* aqueous leaf extract was most effective in correcting damages to liver and kidney tissues caused by the *Salmonella typhimurium* infection.

These findings ascertain the antibacterial activity of *Vernonia amygdalina* leaf extract and its usefulnesss in the treatment of typhoid fever as well as other bacterial diseases. Future studies should try to identify the components of the plant that make it effective in this property.

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