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Research Paper

Hypo-lipidaemic potential and histo-renal changes induced by *Millettia aboensis* in *Salmonella typhi* infected Wistar rats

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ABSTRACT: *Salmonella genus, a name coined after Daniel E. Salmon, the first person to isolate Salmonella enterica serotype Choleraesuis from pig, is a rod-shaped, gram-negative, non-spore-forming, motile entero-bacteria. This study investigated the lipid profile and histo-renal changes linked with Salmonella typhi infectivity in Wistar rats and the potential of ethanol root extract of Millettia aboensis (EREMA) to reverse these changes in comparison with ciprofloxacin. 51 animals were divided into six groups: group 1 was normal control with no treatment but were given feed and water ad libitum, group 2 was infected with Salmonella typhi without treatment (negative control), group 3, 4 and 5 were Salmonella typhi infected and treated with 100mg/kg, 200mg/kg and 400mg/kg of the extract respectively, while group 6 was infected and treated with 7.14mg/kg of ciprofloxacin. The animals were inoculated with a single infectious dose of Salmonella typhi bacterium (2.0 x 10^8cfu/ml) and were subsequently treated with the graded doses of the extract and 7.14mg/kg dose of ciprofloxacin for a period of fifteen days, after the animals were confirmed infected. The rats were humanely sacrificed using diethyl ether anesthesia and blood samples taken for lipid profile assay including [high density lipo-protein cholesterol (HDL-C), low density lipo-protein cholesterol (LDL-C), triglycerides (Trg) and Cholesterol], and the kidney harvested and processed for histological assessment. Inoculation with S. typhi caused a significant decrease in HDL-C and a significant increase in LDL-C, triglycerides (Trg) and Cholesterol and diverse levels of damages to the renal tissues. These were all reversed on treatment with ethanol root extract of Millettia aboensis in a dose-dependent manner. Thus the extract demonstrated both hypo-lipidaemic and histo-renal- curative potentials.*

KEYWORDS: *Chromolaena odorata, Hypo-lipidaemic, histo-renal, Salmonella typhi, Wistar Rats*
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I. INTRODUCTION

Salmonella genus was coined after Daniel E. Salmon, an American veterinarian, the first person to isolate *Salmonella enterica* serotype Choleraesuis, abbreviated as "*S. enterica* serotype Choleraesuis" from pig [1]. *The sub. sp. Enterica*, was explained by Febrega and Vila, [2] as "predominantly motile entero-bacteria, rod-shaped, gram-negative, non-spore-forming, having cell diameters range of 0.7 and 1.5 µm, and lengths of 2 to 5 µm, and peritrichous flagella.'' They are facultative anaerobes, able to survive in the presence or absence of oxygen and showing predominantly peritrichous motility. They lack capsule and are gram-negative rod shaped bacteria, with inability to sporulate [3]. They are characterized by somatic flagellar and surface coated antigen [3].

Salmonella primarily occur in the gastrointestinal tracts of birds, insects, reptiles, and mammals, and environments polluted with human or animal excreta [4]. Primarily it causes typhoid fever in man (the only known natural hosts) [5]. *Salmonella* serovars are flexible bacteria that willingly acclimatize to varieties of harsh ecological circumstances with some growing at temperatures ranging between (2^oC) and (54^oC), with optimal growth temperature range of ($35–37^oC$) [6]. The organisms grows within (4 – 9) pH range with optimal (6.5 – 7.5) pH range and demanding elevated H_2O activity (> 0.94) for development, but also existing at<0.2 H_2O activity, e.g. in dried foods, with the inhibition of growth occurring at temperatures < 7^oC, pH < 3.8 and aw < 0.94 [7].

The mode of entry of *Salmonella* into the host is a direct host cytoskeleton exploitation by *Salmonella* through the injection of a collection of bacterial effector molecules into the host cytoplasm which triggers a dramatic reorganization of the host actin cytoskeleton, resulting in intense membrane gathering and construction of large macro-pinosomes that is in loose contact with the engulfed bacteria [8]. This mechanism is termed 'trigger' mode of entry. The description continued that, the internalized bacteria remain enclosed in a membrane-bound vacuole, called *Salmonella*-containing-vacuole (SCV), which is customized by the bacteria to inhibit fusion with compartments of the lysosomal, or its maturation [8]. This mechanism was further elucidated by Brumell and Grinstein, [9] who demonstrated that "bacteria directed maturation of the *Salmonella*-containing-vacuole leads instead to a development of protected intracellular niche permissive for bacterial replication'', which according to Nasrallah & Nassar, [10], results in extensive internal organs inversion by the organism, most frequently the (bone marrow, spleen & liver) as in histiocytic granulomas, known as typhoid nodules. The inversion of gall bladder or urinary bladder may cause chronic carriage, which is essential for human-to-human transmissions of the disease [10].

In animal research, Solomon *et al*., [11] reported that Salmonella infection caused dyslipidemia in rats with a characteristic enhanced plasma hyper-triglyceridemia, cholesterogenesis and phospholipidosis including a rise in LDL-VLDL and FFA in erythrocytes and plasma. In human studies, Omeh et al. [12], reported an elevation in ALT, AST and ALP levels including LDL-Cholesterol, triacyglycerols, VLDL that is significant statistically and a reduction in HDL, globulin, total protein and albumin concentrations that is significant in typhoid patients in comparison to the normal individuals in a study to examine their serum hepatic biomarkers, lipid profile and kidney status. The research in the therapeutic effect of Propolis in *salmonella typhimirium* infected mice carried out by Preeti *et al.,* [13] revealed that infection with the organism caused less severe histological changes of the kidneys when compared with the organs of the reticulo-endothelial system (spleen and liver). It was demonstrated in this study that the kidney histo-architecture of infected mice revealed extreme infiltration of lymphocytes at higher magnification.

II. METHODS

PLANT COLLECTION

The plant was harvested from natural habitat in Ika community, Akwa-Ibom State, Nigeria in the month of September and Plant roots was identified and authenticated at herbarium unit, in the department of plant science and biotechnology, Faculty of Sciences, University of Port Harcourt, River State, Nigeria with herbarium number UPH/P/104 by Mr. Ekeke Chimezie (Ph.D.)

ISOLATION OF TEST ORGANISMS

The test organism, *S typhi* was isolated from patients with typhoid fever in University of Port Harcourt Teaching Hospital (UPTH), Rivers State. The enrichment media used in course of the isolation of the organism include; strep-tokinase broth [14] and Bile salt broth [15]. The samples presenting perceptible turbidity were sub-cultured on the medium "Mac-Conkey agar". Subsequently, traditional biochemical tests and PCR were used to identify the isolates exhibiting specific colonies

EXTRACTION METHOD

The bark of the root of the plant were shredded out using cutlass, washed with clean tap water and allowed to dry at room temperature between 32-35^{o}C, until they attained a constant weight. The extraction method used was adapted from Hanan *et al*., [16] cold maceration extraction protocol, with diminutive adjustment. The powdered *M aboensis* root bark of about 50g was soaked in 70% ethanol of about 1000ml in a 2 litre flask and mixed forcefully at 1hr intermission, for 12 hrs and allowed to settle over-night (35^{o}C) to allow for adequate extraction. Subsequently, the concoction was filtered by means of a filter paper with pore size of 0.45milli-pore. The concentration of the extract was increase using rotary evaporation process at 40^{o}C and 200 rpm. The final semi-solid extract was obtained by drying the content of the rotary evaporator over a steam bath at 40^{o}C. The resultant extract obtained 20% yield, was kept safe at room temperature in desiccators, until it was needed for the study.

EXPERIMENTAL DESIGN

Fifty one (51) animals were separated into 6 groups. Group 1 (normal) had three (3) animals, Group 2 (negative control) had twelve (12) animals, while groups 3-6 each had nine (9) animals. Group 1 animals were not treated throughout the experimental period but were given free access to normal animal feed and water *ad labitum*. Group 2 contained *Salmonella typhi*-infected rats not treated after disease induction. Group 3 contained *Salmonella typhi*-infected rats treated with100mg/kg (low dose) of *Milletia aboensis* root extract. Group 4 contained *Salmonella typhi*-infected rats treated with 200mg/kg (medium dose) of ethanol root extract of *Milletia aboensis*. Group 5 contained *Salmonella typhi*-infected rats treated with 400mg/kg (high dose) of

ethanol root extract of *Milletia aboensis*. Group 6 contained *Salmonella typhi*-infected rats treated with 500mg/70kg (7.14mg/kg) of a standard antibiotic drug (Ciprofloxacin).

On day 0, (when the animals were confirmed infected, through observation of anorexia, weakness and diarrhea as well as isolation of the organism from the animal stool), and at six day intervals and on day sixteen, 3 animals from each group were humanely sacrificed and blood was collected for lipid profile evaluation and the kidneys removed for histopathological assessment.

CHALLENGING APPARENTLY HEALTHY ANIMALS WITH *SALMONELLA TYPHI*

Forty eight (48) animals (groups 2-6) were orogastrically challenged with an infective dose (2.0 x 10^8cfu/ml) of *Salmonella typhi*. After infection had set in (through observation of signs like weakness, anorexia, non-productive cough, watery stool, standing of the hairs as in cold condition and isolation of the organism from the animal stool) (day 0), three animals were sacrificed and blood samples and kidney tissues collected for preliminary screening while the other 45 animals were treated with the ethanol extract of *Milletia aboensis* according to the different doses and the standard antibiotic (Ciprofloxacin), once daily, for fifteen days.

PREPARATION OF THE EXTRACT CONCENTRATIONS AND ANTIBIOTIC

Stock solution for the extract was prepared by dissolving 500 mg in 1 ml of sterile distilled water. An antibiotic control was made by dissolving 500mg of ciprofloxacin in sterile distilled water.

BLOOD COLLECTION AND DISSECTION

Blood was collected from each animal by cardiac puncture method after the animals were anaesthetized with diethyl ether in a desiccator. The blood was immediately transferred into appropriately labelled sample bottles containing anticoagulant and the kidney was removed aseptically and was weighed and a portion was kept for histological analysis.

LIPID PROFILE ANALYSIS

Cholesterol and High Density Lipoprotein-Cholesterol (HDL) determination were carried out using Randox automated method and the method of Lothar [17], while Triglycerides (Trigs) determination was carried out using Randox automated method and the method of Tietz [18].

HISTOPATHOLOGY STUDIES

The animals were anaesthetized with diethyl ether, dissected aseptically to remove the kidneys which were then transferred into 10% chloroform and later trimmed down to a size between 2mm to 4mm thickness, to allow the fixative to readily penetrate the tissue. The tissues were exposed to different stages of processing by standard methods as described by Baker [19], including, fixation, dehydration, clearing, impregnation, embedding, sectioning and staining with hematoxylin and eosin (H&E) and finally mounting.

STATISTICAL ANALYSIS

The results are presented as Mean ± Standard error of mean. Differences between means were assessed using Analysis of variance (ANOVA) and post test using LSD multiple comparison test [20].

III.RESULTS

Salmonella typhi inoculation significantly ($P<0.05$) lowered the plasma level of HDL and elevated the concentrations of Total cholesterol, triglycerides and LDL. These changes were reversed by ethanol root extract of *Milletia aboensis* and Ciprofloxacin, leading to a gradual elevation in HDL levels and reduction in triglycerides, cholesterol and LDL levels. The differences between normal and negative control, and between treatment groups and negative control in LDL, HDL and triglyceride concentrations were significant ($P<0.05$) on the 6^{th}, 11^{th}& 16^{th} day, as presented in figures 1, 2, and 3. The changes in cholesterol levels were not significant (figure 4). T

he kidney histo-pathological examination of the control animals showed normal glomeruli properly placed in the cortex of the tissue. Bowman's space was not distended and no cellular alterations were observed (plate 1), while those with *S. typhi* infection without treatment showed inflammatory cells and edematous tubules (nephritis) (plate 2).

The kidneys of *S. typhi* infected rats treated for 5 days with 100mg/kg of ethanol root extract of *Milletia aboensis* showed glomerular with intense Bowman's capsule with loss of pigmentation in the tissue showing mild edematous degeneration (plate 3), those treated with 200mg/kg for 5 days showed that the renal adipose tissue had interlobar arteries, while the intact presence of the perinephric adipose tissue did not indicate any pathology (plate 4), and those treated with 400mg/kg for 5 days showed normal kidney glomeruli tissue

surrounding tubules with no form of necrosis or inflammation (plate 5), while those treated with 500mg/70kg of ciprofloxacin for 5 days showed normal glomeruli without tubular necrosis or inflammation (plate 6).
Kidney tissues of rats infected with *S. typhi* and treated with 100mg/kg of *M. aboensis*for 10 days, showed glomeruli edematous tubules (nephritis) (plate 9); for kidney tissues of rats infected with *S. typhi* and treated with 200mg/kg of *M. aboensis* for 10 days, showing inflamed kidney tubules with loss of its cellular architecture without necrosis (plate 10), while kidney tissues of rats infected with *S. typhi*, treated with 400mg/kg of *M. aboensis* for 10 days, showed normal and intact glomerulus without necrosis (plate 11). Similarly, kidney tissues of rats infected with *S. typhi* and treated with 100mg/kg, 200mg/kg and 400mg/kg of *M. aboensis*for 15 days, showed normal kidney tissues. They are shown in plates 15, 16 and 17 respectively.

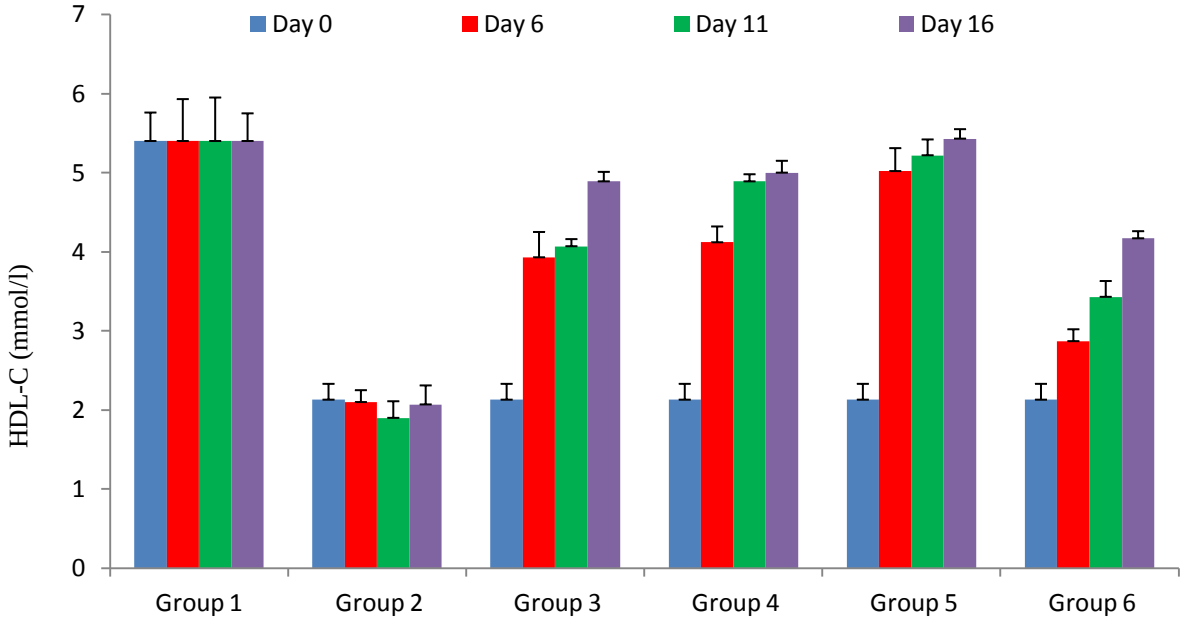

Figure 1: High Density Lipoprotein Cholesterol (HDL-C) of Albino Rats exposed to Salmonella typhi bacteria before treatment with Milletia aboensis

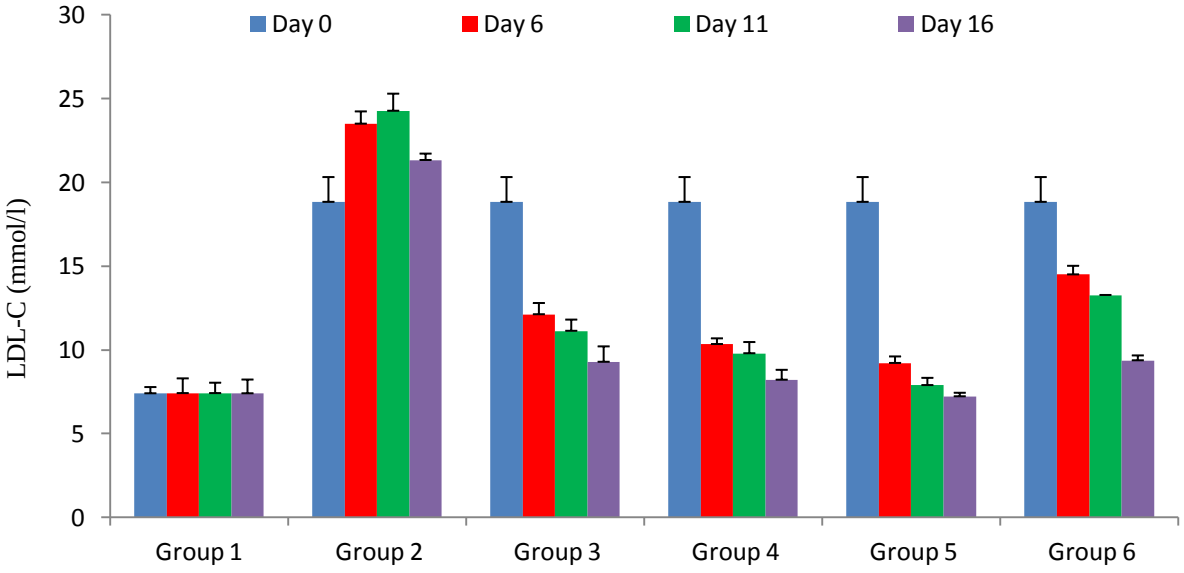

Figure 2: Low Density Lipoprotein Cholesterol (LDL-C) of Albino Rats exposed to Salmonella typhi bacteria before treatment with Milletia aboensis

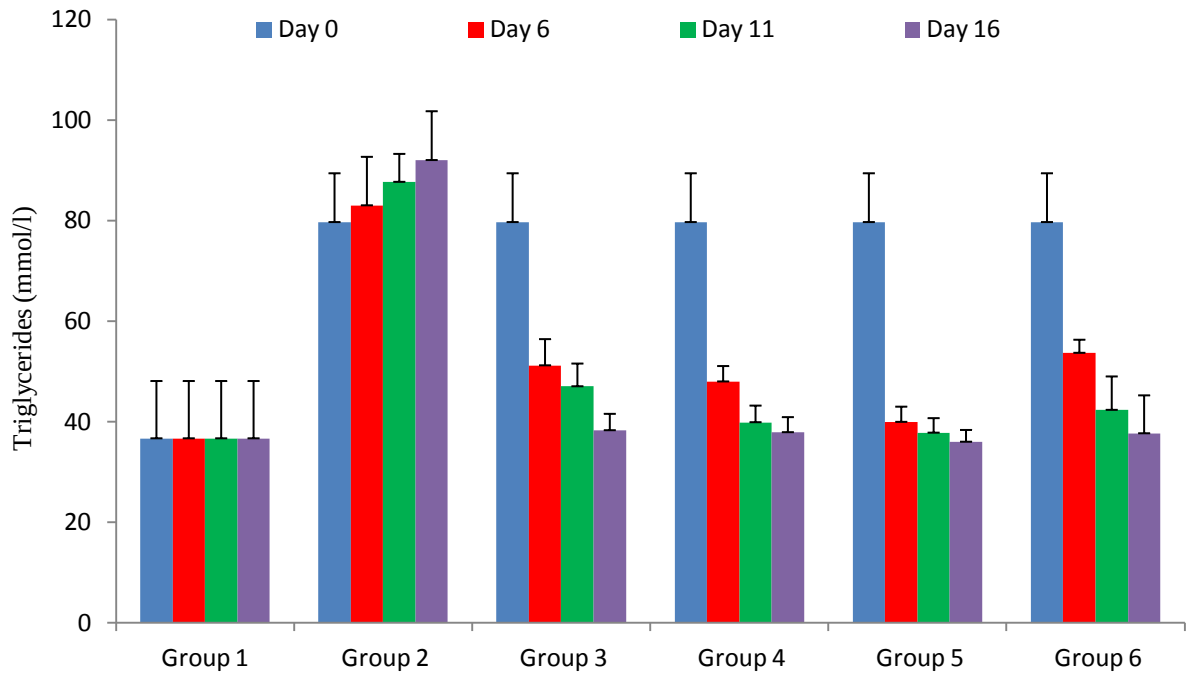

Figure 3: Triglycerides of Albino Rats exposed to Salmonella typhi bacteria before treatment with Milletia aboensis

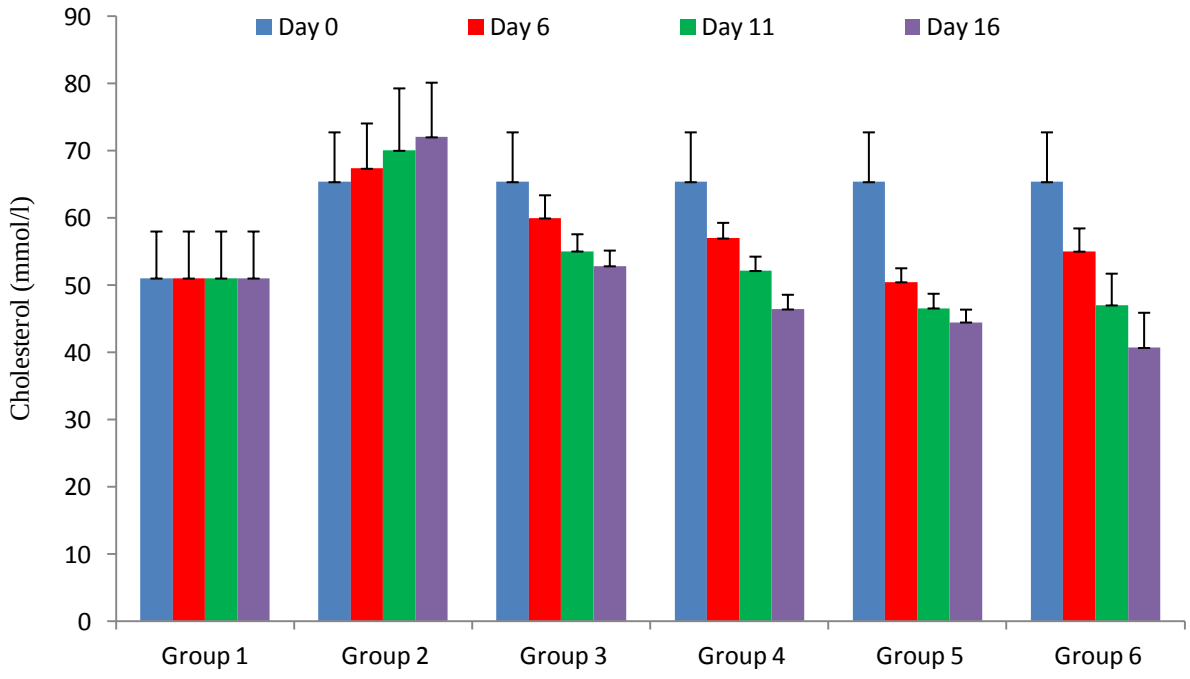

Figure 4: Cholesterol of Albino Rats exposed to Salmonella typhi bacteria before treatment with Milletia aboensis

HISTOLOGY OF THE KIDNEY

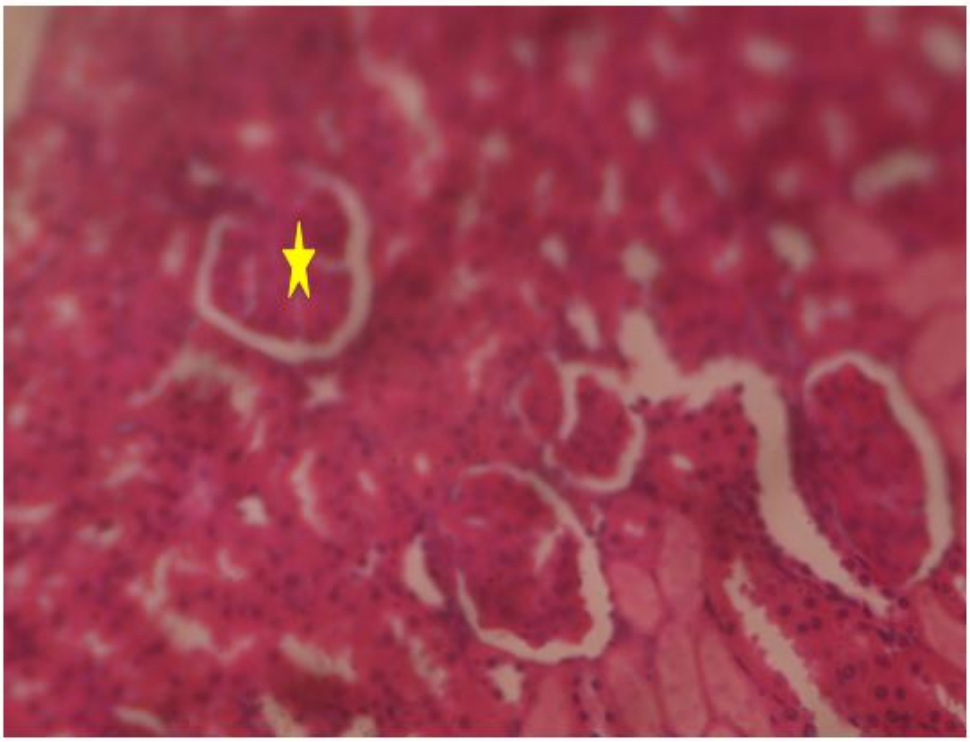

Plate 1: Photomicrograph of kidney tissues of normal rats (group one) after 5 days of study, showing that the kidney had normal glomeruli (as shown by the star shape) properly placed in the cortex of the tissue with non-distension of the Bowman's space as well as without cellular alterations

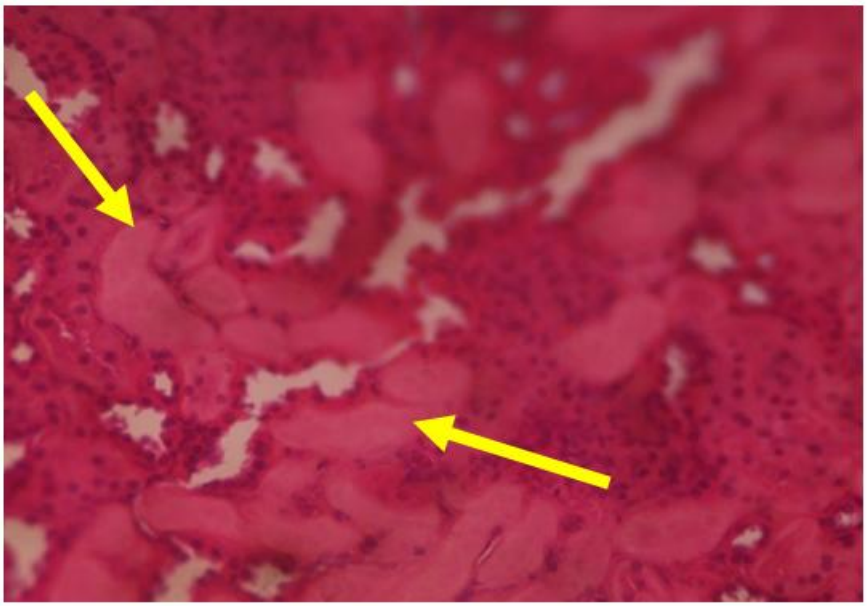

Plate 2: Photomicrograph of kidney tissues of rats infected with *S. typhi*, without treatment (group two) for 5 days showing inflammatory cells and edematous tubules (nephritis) as shown by the arrows.

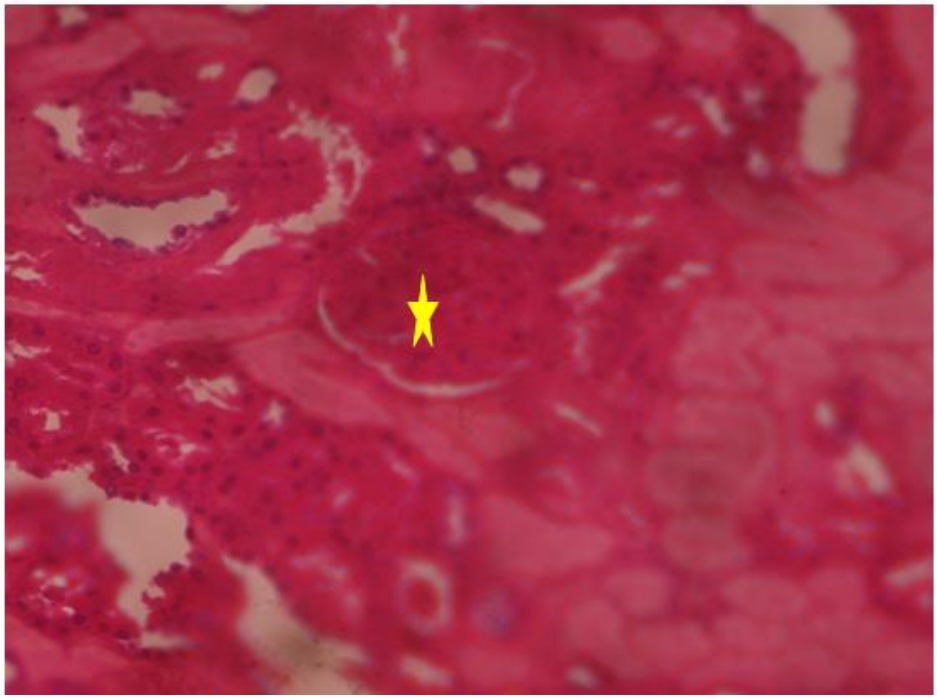

Plate 3`: Photomicrograph of kidney tissues of rats infected with *S. typhi* and treated with 100mg/kg of *M. aboensis* (group four) for 5 days, showing glomerular with intense Bowman's capsule with loss of pigmentation in the tissue showing mild edematous degeneration (see star shape)

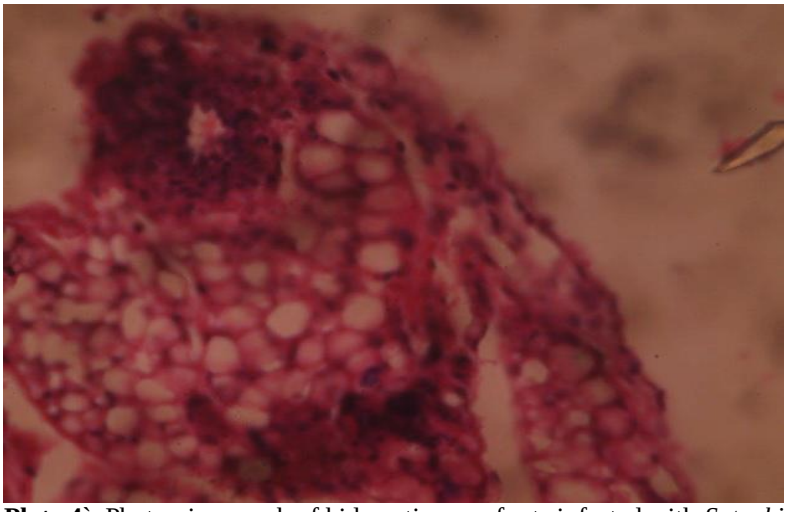

Plate 4`: Photomicrograph of kidney tissues of rats infected with *S. typhi* and treated with 200mg/kg of *M. aboensis* (group three) for 5 days, showing that the renal adipose tissue pictured in the diagram also had the presence of interlobar arteries. The intact presence of the perinephric adipose tissue does not indicate any pathology.

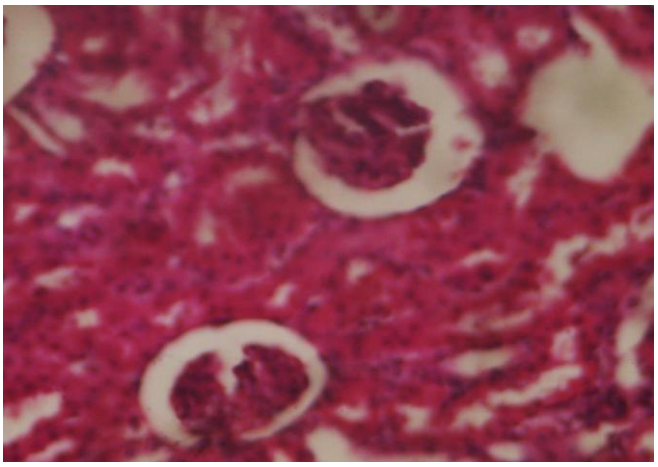

Plate 5`: Photomicrograph of kidney tissues of rats infected with *S. typhi* and treated with 400mg/kg of *M. aboensis* (group four) for 5 days, showing normal kidney tissue glomeruli and surrounding tubules with no form of necrosis or inflammation

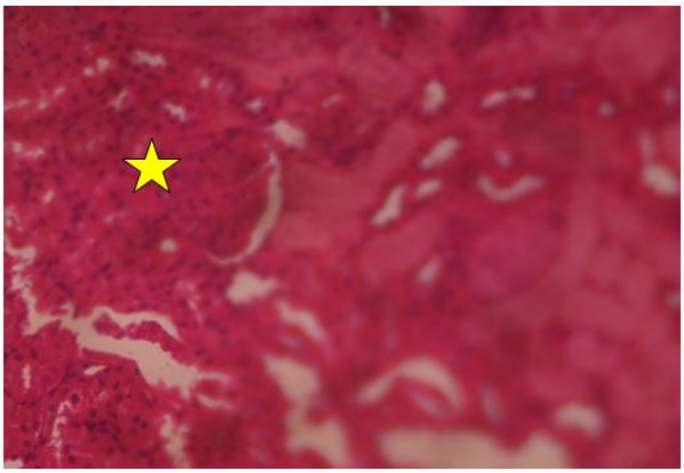

Plate 6: photomicrograph of kidney tissues of *S. typhi* infected rats treated with 7.14mg/kg of *Ciprofloxacin* (group six) for 5 days, showing that the glomeruli in the kidney were rich in polymorphonuclear cells as shown by the star shape. The surrounding trabecular though partly differentiated did not show any sign of necrosis.

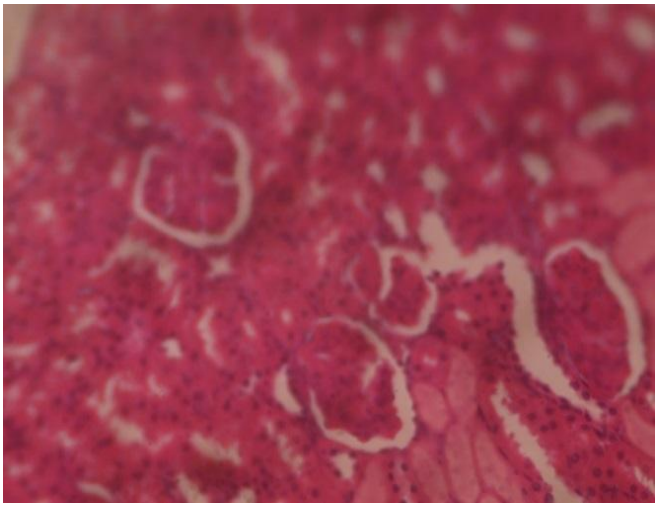

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Plate 7: Photomicrograph of kidney tissues of normal rats (group one) after 10 days of study, showing that the kidney had normal glomeruli (as shown by the star shape) properly placed in the cortex of the tissue with non-distension of the Bowman's space as well as without cellular alterations

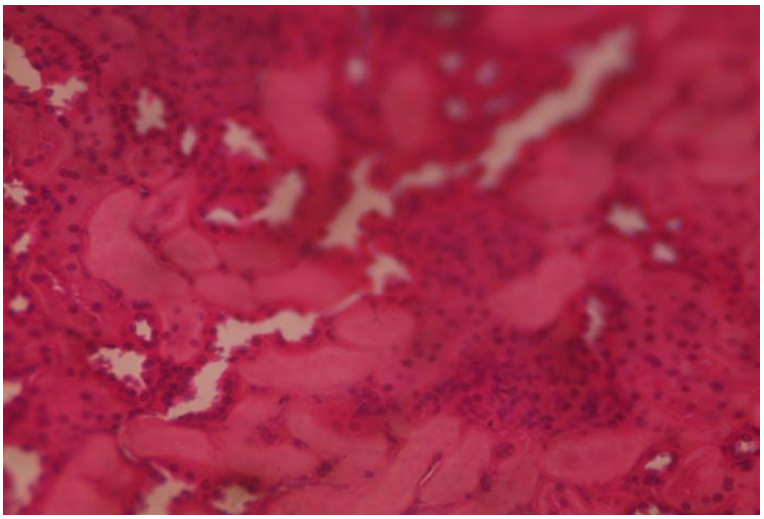

Plate 8: Photomicrograph of kidney tissues of rats infected with *S. typhi*, without treatment (group two) for 10 days showing that the kidney was replete with inflammatory cells and edematous tubules (nephritis)

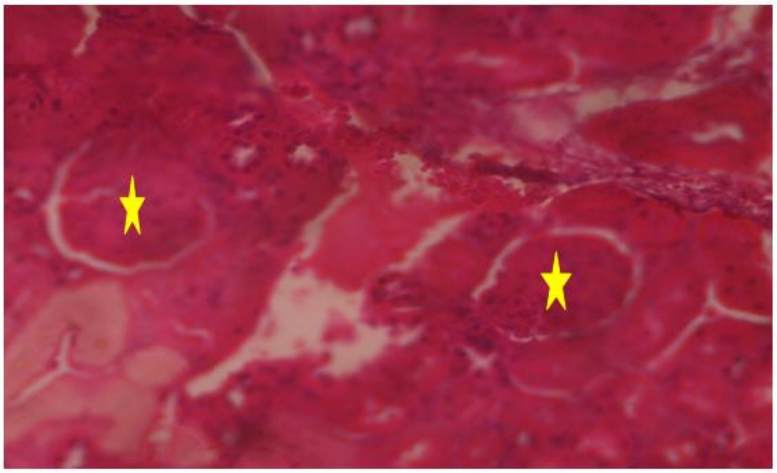

Plate 9: Photomicrograph of kidney tissues of rats infected with *S. typhi* and treated with 100mg/kg of *M. aboensis* (group four) for 10 days, showing glomeruli edematous tubules (nephritis)

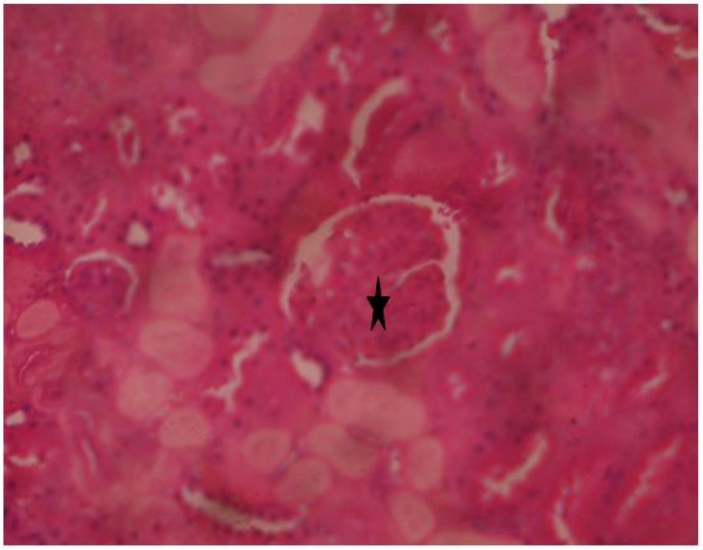

Plate 10: Photomicrograph of kidney tissues of rats infected with *S. typhi* and treated with 200mg/kg of *M. aboensis* (group three) for 10 days, showing Inflamed kidney tubules with loss of its cellular architecture without necrosis

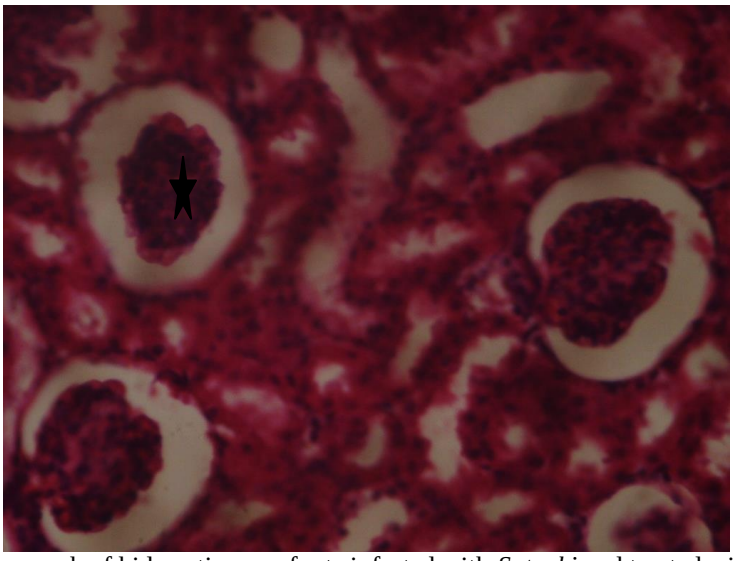

Plate 11: Photomicrograph of kidney tissues of rats infected with *S. typhi* and treated with 400mg/kg of *M. aboensis* (group four) for 10 days, showing Normal and intact glomerulus without necrosis

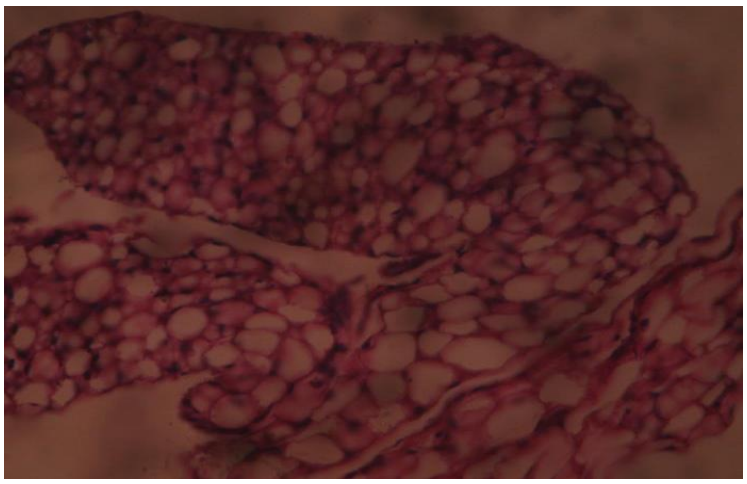

Plate 12: photomicrograph of kidney tissues of *S. typhi* infected rats treated with 7.14mg/kg of *Ciprofloxacin* (group six) for 10 days, showing renal adipose tissue with intact presence of the perinephric adipose tissue not indicating any pathology

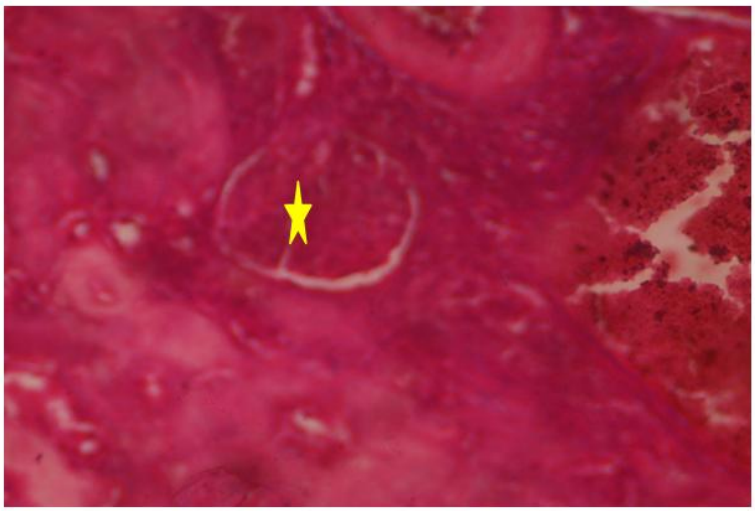

Plate 13: Photomicrograph of kidney tissues of normal rats (group one) after 15 days of study, showing well defined kidney tissues

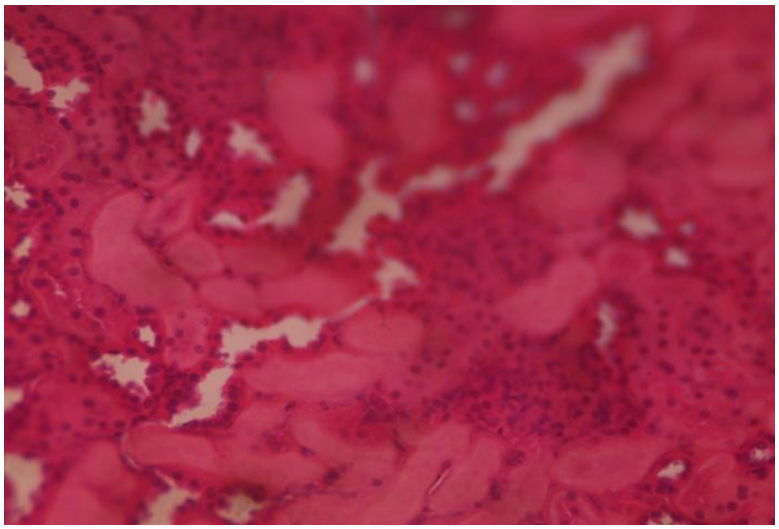

Plate 14: Photomicrograph of kidney tissues of rats infected with *S. typhi*, without treatment (group two) for 15 days showing that the kidney was replete with inflammatory cells and edematous tubules (nephritis)

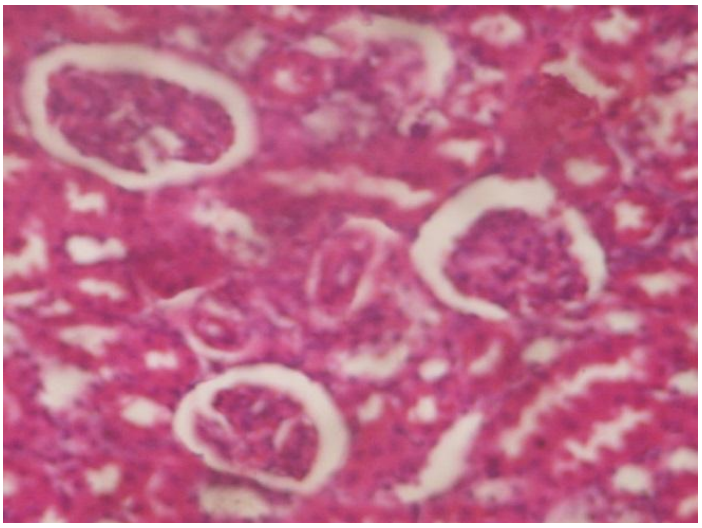

Plate 15: Photomicrograph of kidney tissues of rats infected with *S. typhi* and treated with 100mg/kg of *M. aboensis* (group four) for 15 days, showing normal kidney tissues

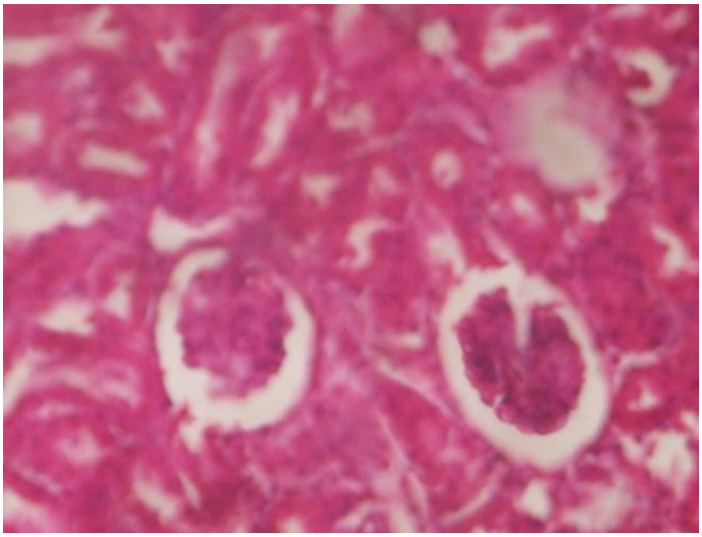

Plate 16: Photomicrograph of kidney tissues of rats infected with *S. typhi* and treated with 200mg/kg of *M. aboensis* (group three) for 15 days, showing normal kidney tubules cellular architecture without necrosis

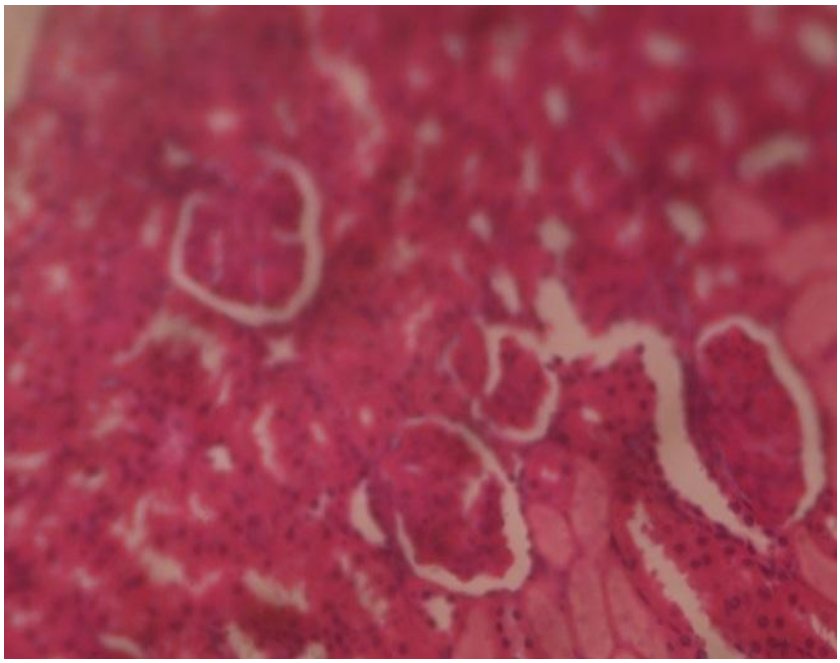

Plate 17: Photomicrograph of kidney tissues of rats infected with *S. typhi* and treated with 400mg/kg of *M. aboensis* (group four) for 15 days, showing Normal and intact glomerulus without necrosis

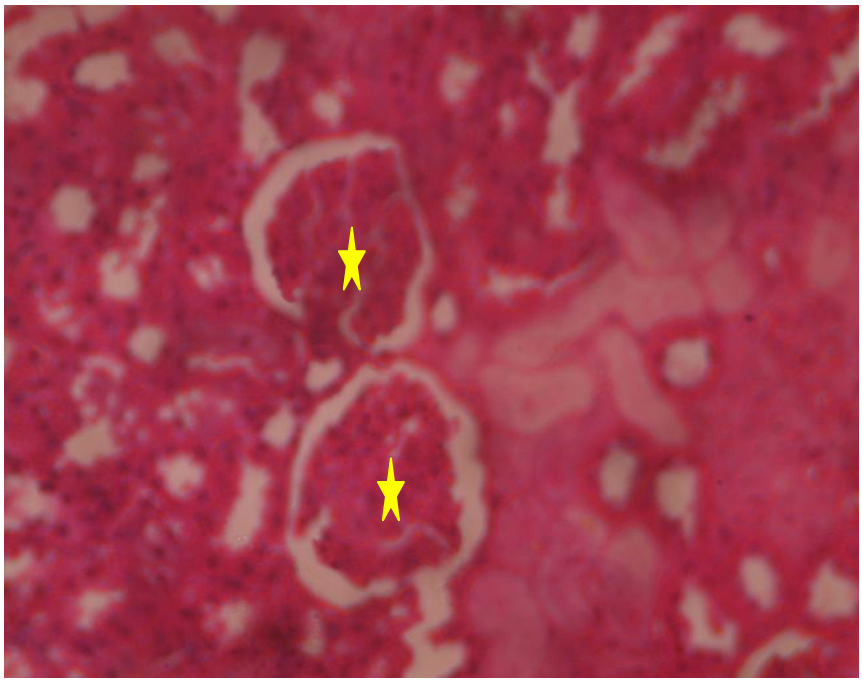

Plate 18: photomicrograph of kidney tissues of *S. typhi* infected rats treated with 7.14mg/kg of *Ciprofloxacin* (group six) for 15 days, showing kidney tissues with the presence of normal glomeruli and Bowman's capsule (see star shapes).

IV.DISCUSSION

S. typhi infections always lead to an increase in serum levels of; Cholesterol, Triglycerides and Cholesterol, as well as an associated decrease in HDL [21]. Our findings also agree with these reports. These effects may be due to inhibition of LDL clearance from the circulatory system [22]. stimulation of VLDL production by raising the hepatic de novo fatty acid production, increasing adipose tissue lipolysis, and reducing hepatic fatty acid oxidation [23] and [24]. Treatment of the infected rats with ethanol root extract of *Milletia aboensis* reversed the usual significant increase ($p<0.05$) in Cholesterol, Triglycerides and LDL as well as the usual significant decrease ($p<0.05$) in HDL associated with *S.typhi* infection. The reversion of abnormal lipid parameters agrees with the findings of Onyegeme-Okerenta and Essien [25]. Thus the extract may have

produced these effects by either, enhancing LDL-C clearance, preventing VLDL denovo synthesis, decreasing adipose tissue lipolysis and enhancing fatty acid oxidation. These observations revealed potential hypo-lipidaemic, as well as cardio-protective effect of the extract against degenerative diseases [26] and anti-*Salmonella typhi* effects which is in agreement with the report of Blessing and Uzoma [27], who found *Millettia aboensis* to have exhibited significant antibacterial activity against clinical Isolates of *P. Aeruginosa, S. aureus,* and *K. Pneumonia*. Kidney of rat infected with *S. typhi* showed inflammatory cells and edematous tubules (nephritis) and necrosis, as has been explained by Nishiura *et al.*, [28] that these are among signs of disease manifestation. The appearance of nephrotic loci tubular necrosis is explained by Ajibade and Famurewa, [29] to reflect injury to renal tubular epithelial cells, thus the reversal of these changes by ethanol root extract of *Millettia aboensis* also suggests an anti-Salmonella typhi activity.

V. CONCLUSION

Inoculation of Wistar rats with *Salmonella typhi* resulted in an increase in LDL, Triglycerides and Cholesterol as well as a decrease in HDL, while administration of ethanol root extract of *Millettia aboensis* to the infected animals caused a decrease in LDL, Triglycerides and Cholesterol as well as an increase in HDL. Also, *Salmonella typhi* infection caused inflammatory cells and edematous tubules (nephritis) and necrosis, while administration of the extract reversed these changes.

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