Evidence of Hepato-Curative Potentials of *Millettia aboensis* in *Salmonella typhi* infected Wistar Rats

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Abstract: Typhoid fever is a systemic infection caused by the bacterium *Salmonella enterica* subspecies *enterica* serotype *typhi*, a foremost public health predicament in developing countries. This study investigated hepatotoxicological changes associated with *Salmonella typhi* infection in Wistar rats and the potential of ethanol root extract of *Millettia aboensis* (EREMA) to reverse these changes. 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, and 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 (2.0×10^8 cfu/ml) and were subsequently treated with the graded doses of the extract and 7.14mg/kg of ciprofloxacin for a period of fifteen days. The rats were humanely sacrificed using diethyl ether anesthesia and blood samples taken for liver function investigation including [aspartate aminotransferase (AST) alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), Albumin, conjugate bilirubin (CB) and total protein (TP)] and liver harvested and processed for histological assessment. Inoculation with *S. typhi* caused significant increase in all the liver function parameters and different degrees of damages to the liver cells. These were all reversed on treatment with ethanol root extract of *Millettia aboensis*. Thus the extract exhibited both anti-*Salmonella typhi* as well as hepato-curative potentials on the Kuffer cells.

Keywords: *Chromolaena odorata*, Hepato-curative, *Salmonella typhi*, Wistar Rats

Introduction

Typhoid fever is a systemic infection caused by the bacterium *Salmonella enterica* subspecies *enterica* serotype *typhi* (Iroha *et al.*, 2010). It constitutes a foremost public health predicament in many developing nations of the world (Crump, *et al.*, 2004). Typhoid fever principally attacks children and young adults and is known to be a major cause of global morbidity with over 600,000 deaths annually (Abro *et al.*, 2009 and Wasfy *et al.*, 2000). *Salmonella typhi* is transmitted via the faecal-oral route, both directly from person to person or by ingestion of food or water contaminated with faeces (Ivanoff, 1995). The characteristics of the disease in its early stage include high fever, anorexia, colic pain, malaise, lethargy, diarrhea and dull continuous headache. During advanced stages of the disease, it is indicated by mental dullness and protracted fever, while other symptoms may include slight deafness, intestinal bleeding, and parotitis paratyphoid (Ackers, 2000). Typhoid fever usually is connected with hepatic pathology, even though, Abro *et al.*, (2009) showed that rigorous liver injury causing delicate viral hepatitis is rare. Although, hepatitis patho-genesis is unclear, liver cells destruction in

typhoid related illness has various mechanisms involving regional or general adverse activities of specific endotoxin, unspecified inflammatory response to gastro-intestinal injury together with cyto-toxins produced and discharged by *S. typhi* in Kupffer cells (Abro *et al.*, 2009). The regular occurrence of hepatic enzyme in typhoid fever was shown to be (22%, 26% and 52%) in different situations (Morgenstern & Hayes, 1991 and Mirsadraee *et al.*, 2007), while Ozen in 1995, reported that typhoid fever disease is associated with abnormal liver function tests in about 50% of patients, similarly, Abro *et al.*, (2009) also described a more regular occurrence for alanine transaminase in typhoid patients in proportions of (73.3%). In a research to treat *S. typhi* infection in rats using *Momordica charatia*, Adeyi, et al., (2013), found that infection with *S. typhi* resulted in a rise in the levels of Gamma-glutamyl-transferase, ALP, AST, ALT and TB. Also in a similar study by Isirima and Siminialayi (2018), to treat *S. typhi* infection in Wistar rats with *Chromolaena odorata*, it was reported that inoculation of Wistar rats with *Salmonella typhi* caused an increase in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), albumin and total protein (TP), and an insignificant increase in Conjugated Billirubin (CB) as well as hepatic necrosis on histological examination. The World Health Organization (WHO) reported that more than 80% of the world's population depends on traditional medicine for their principal healthcare needs, and a larger proportion of this comes from plants sources or their active principles (Gupta *et al.*, 2005). Plants used in traditional medical practice contain an extensive variety of ingredients that can be used to treat both infectious and chronic diseases (Iroha *et al.*, 2010). According to (Sarkiyay *et al.*, 2011), microbial infections poses severe health crisis in the entire globe and that plants are possible source of antimicrobial agents. (Sarkiyay *et al.*, 2011) equally reported that medicinal plants contain active principles which could serve as substitute for inexpensive and valuable herbal drugs against frequent bacterial infections. *Millettia aboensis* occur in the family of Fabaceae. It is frequently called 'Uturuekpa' in South Eastern part of Nigeria. The plant is a small tree of about 30–40 feet tall, and up to 2 feet in thickness, usually dressed with reddish-brown pubescence on the inflorescence, branches, fruits and petioles (Adonu *et al.*, 2013). The plant is usually found in low land rain forest, with evidently beautiful purple flowers in erect terminal racemes at branches and rusty-hairy leaves (Uba 2010). The aqueous and ethanol extracts of the plant have been reported to possess hepatoprotective potentials against Paracetamol induced hepatotoxicity, reversing liver enzymes (AST, ALT, ALP and serum total and conjugated Bilirubin) near to normal levels in a concentration dependent manner (Attama *et al.*, 2014).

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 (Watson, 1978) and Bile salt broth (Watson, 1954). 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°C, until they attained a constant weight. The extraction method used was adapted from Hanan *et al.*, (2013) 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°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°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°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 libitum*. Group 2 contained *Salmonella typhi*-infected rats not treated after disease induction. Group 3 contained *Salmonella typhi*-infected rats treated with 100mg/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 from the animals 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 and the liver removed for assessment of the liver function parameters and histopathological examination, respectively.

Challenging apparently healthy animals with *Salmonella typhi*

Forty eight (48) animals (groups 2-6) were orogastrically challenged with an infective dose (2.0×10^8 cfu/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 liver 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 liver was removed aseptically and was weighed and a portion was kept for histological analysis.

Liver Function Test Analysis

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) determinations were carried out using Randox automated method and the methods of Reitman & Frankel (1957) and Schmidt, & Schmidt, (1963), while alkaline phosphatase (ALP) determination was carried out using Randox automated method and the methods of Klein, & Babson, (1960) and Babson, et al., (1966). In a similar manner total protein (TP) determination was carried out using Randox automated method and the method of Tietz (1995), while albumin (ALB) and bilirubin (BIL) determinations were carried out using Randox automated method and the methods of Grant (1987) and Dumas, et al., (1971).

Histopathology Studies

The animals were anaesthetized with diethyl ether, dissected aseptically to remove the liver which was 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 (1945), 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 $\pm$ Standard error of mean. Differences between means were assessed using Analysis of variance (ANOVA) and post test using LSD multiple comparison test (Mead, & Curnow, 1982).

Results

Effect of ethanol root extract of *Milletia aboensis* on liver function parameters in *S. typhi* infected Wistar rats

Inoculation with only one infective dose of *S. typhi* to rats showed a significant elevation ($p < 0.05$) in TP, TB, ALP, ALT, AST and albumin levels apart from CB which recorded an insignificant change when compared to normal control. On the contrary, treatment with ethanol root extract of *Milletia aboensis* (EREMA) and ciprofloxacin reversed the observed elevations, producing a stable reduction across the parameters on days 11th and 16th days as shown in figures 1, 2, 3, 4, 6, and 7, as well as a significant difference ($p < 0.05$) between treatment groups and negative control on the 16th day in CB as presented in figure 5.

Effect of ethanol root extract of *Milletia aboensis* on liver histology in *Salmonella typhi* infected Wistar rats

Histological assessment of hepatic tissues from un-treated rats demonstrated normal hepatocytes histo-architecture but harsh necrosis was noted in untreated *S. typhi* infected rats. For rats infected and administered with 100mg/kg of ethanol root extract of *Milletia aboensis* for 5 days, it was observed that there was a high level of inflammatory polymorphonuclear cells, with the portal triad showing hepatic artery and vein as indicated by the arrow I plate 3; while animals treated with 200mg/kg of extract for 5 days revealed, liver with a prominent central vein (as indicated by the arrow in plate 4), with readily placed hepatic plates and well dilated sinusoids. Rats treated with 400mg/kg of extract for 5 days showed liver with central veins from the liver lobules in proper position indicating normal architectural appearance (plate 5). The histology of the liver in animals treated with 100mg/kg, for 10 days revealed poorly differentiated liver cells although with well dilated sinusoids and the presence of inflammatory cells (plate 9); while for 200mg/kg for 10 days showed hepatic lobule containing hepatic plates that are radially placed with dilated sinusoids and marked interlobar region containing branches of hepatic arteries and portal vein without inflammatory cells (plate 10); and for those treated with 400mg/kg for 10 days showed normal hepatocytes and central vein (plate 11). Also among the histological tissues of the liver cells treated with 100mg/kg, 200mg/kg and 400mg/kg, for 15 days only those with 400mg/kg revealed normal hepatocytes. These are shown in plates 15, 16 and 17 respectively. The hepatic histo-architecture following infection and a five days course of treatment with Ciprofloxacin (500mg/70kg), showed no disrupted tissues and portal triad as shown in plate 6 and normal sinusoids without inflammation as well as sinusoids with clear hepatic plates devoid of any inflammatory cells after 10 days therapy (plate 12), while animals treated for 15 days with Ciprofloxacin (500mg/70kg/kg) showed inter-lobular section having crowded inter-lobular portal vein.

Figure 1: Aspartate aminotransferase (AST) of Albino Rats exposed to Salmonella typhi bacteria before treatment with Millettia aboensis

Figure 2: Alanine aminotransferase (ALT) of Albino Rats exposed to Salmonella typhi bacteria before treatment with *Milletia aboensis*

Figure 3: Alkaline Phosphatase (ALP) of Albino Rats exposed to Salmonella typhi bacteria before treatment with Milletia aboensis

Figure 4: Total Bilirubin (TB) of Albino Rats exposed to Salmonella typhi bacteria before treatment with Millettia aboensis

Figure 5: Conjugate Bilirubin (TB) of Albino Rats exposed to Salmonella typhi bacteria before treatment with Millettia aboensis

Figure 6: Albumin levels of Albino Rats exposed to Salmonella typhi bacteria before treatment with Millettia aboensis

Figure 7: Total Proteins (TP) level of Albino Rats exposed to Salmonella typhi bacteria before treatment with Millettia aboensis

Plate 1: Photomicrograph of liver tissues of normal rats (group one) after 5 days of study, showing normal architecture with prominent central vein as indicated with the arrow

Plate 2: Photomicrograph of liver tissues of rats infected with *S. typhi*, without treatment (group two) for 5 days showing liver necrosis with poorly defined architecture as shown by the arrow.

Plate 3`: Photomicrograph of liver tissues of rats infected with *S. typhi* and treated with 100mg/kg of *M. aboensis* (group three) for 5 days, showing high level of inflammatory polymorphonuclear cells, with the portal triad presenting a hepatic artery and vein as indicated by the arrow

Plate 4`: Photomicrograph of liver tissues of rats infected with *S. typhi* and treated with 200mg/kg of *M. aboensis* (group four) for 5 days, showing liver with a prominent central vein (as indicated by the arrow), with readily placed hepatic plates and well dilated sinusoids.

Plate 5`: Photomicrograph of liver tissues of rats infected with *S. typhi* and treated with 400mg/kg of *M. aboensis* (group four) for 5 days, showing liver with central veins from the liver lobules in proper position indicating normal architectural appearance.

Plate 6: photomicrograph of liver tissues of *S. typhi* infected rats treated with 7.14mg/kg of *Ciprofloxacin* (group six) for 5 days, showing well dilated sinusoids with no inflammatory cells with intense portal triad branching of the hepatic artery and vein as indicated with the circular shape

Plate 7: Photomicrograph of liver tissues of normal rats (group one) after 10 days of study, showing normal architecture with radially placed central vein and hepatic plates

Plate 8: Photomicrograph of liver tissues of rats infected with *S. typhi*, without treatment (group two) for 10 days showing liver partly necrotized with poorly defined architecture as shown by the arrow.

Plate 9`: Photomicrograph of liver tissues of rats infected with *S. typhi* and treated with 100mg/kg of *M. aboensis* (group three) for 10 days, showing poorly differentiated liver cells although with well dilated sinusoids and the presence of inflammatory cells.

Plate 10`: Photomicrograph of liver tissues of rats infected with *S. typhi* and treated with 200mg/kg of *M. aboensis* (group four) for 10 days, showing liver lobule containing hepatic plates that are radially placed with dilated sinusoids and marked interlobar region containing branches of hepatic arteries and portal vein without inflammatory cells (see circular figure)..

Plate 11` : Photomicrograph of liver tissues of rats infected with *S. typhi* and treated with 400mg/kg of *M. aboensis* (group five) for 10 days, showing normal hepatocytes and central vein.

Plate 12: photomicrograph of liver tissues of *S. typhi* infected rats treated with 7.14mg/kg of *Ciprofloxacin* (group six) for 10 days, showing well defined hepatic plates with no presence of inflammatory cells.

Plate 13: Photomicrograph of liver tissues of normal rats (group one) after 15 days of study, showing normal architecture with radially placed central vein and hepatic plates

Plate 14: Photomicrograph of liver tissues of rats infected with *S. typhi*, without treatment (group two) for 15 days showing liver partly necrotized with poorly defined architecture as shown by the arrow.

Plate 15: Photomicrograph of liver tissues of rats infected with *S. typhi* and treated with 100mg/kg of *M. aboensis* (group three) for 15 days, showing interlobe junction of the hepatic tissue with the presence of portal veins and arteries (as indicated by the star shape)

Plate 16: Photomicrograph of liver tissues of rats infected with *S. typhi* and treated with 200mg/kg of *M. aboensis* (group four) for 15 days, showing intact architecture with central vein containing blood cells marked by the arrow

Plate 17: Photomicrograph of liver tissues of rats infected with *S. typhi* and treated with 400mg/kg of *M. aboensis* (group four) for 15 days, showing intact and normal architecture.

Plate 18: photomicrograph of liver tissues of *S. typhi* infected rats treated with 7.14mg/kg of *Ciprofloxacin* (group six) for 15 days, showing well radially placed central vein and hepatic plates and well dilated sinusoids and some Kupfer cells were seen in the sinusoidal place.

Discussion

In this study it was observed that inoculation of Wistar rats with *Salmonella typhi* caused an increase in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), albumin and total protein (TP) and an insignificant increase in Conjugate Billirubin (CB). These findings are in line with the reports of Adeyi *et al.*, (2013) and Damilola *et al.*, (2015) and Isirima *et al.*, (2018). These changes as remarked by Sallie *et al.*, (1991) may have occurred due to hepatic damage resulting from local or systemic adverse effects of particular endotoxin, non-specific inflammatory reactions in response to intestinal perforations and cytotoxins generated and released by *Salmonella typhi* that have infected Kuffer cells. It was also observed that administration of ethanol root extract of *Milletia aboensis* reversed the adverse hepatotoxicological changes and this observation also agrees with the findings of Attama *et al.*, (2014), who also reported a reduction in liver and kidney markers including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum total and conjugated Bilirubins on administration of *Milletia aboensis* root extract extract to Wistar rats. These reversal effects could be hepatoprotective in nature. These effects could be attributed to anti-bacterial potentials of *Milletia aboensis* by eliminating the bacteria from the Kuffer cells. Infection of Wistar rats resulted in severe hepatic necrosis, which agrees with the reports of Haque, *et al.*, (2011) and Isirima *et al.*, (2018). Such liver pathology has been attributed to liver disease (Mohd *et al.*, 2013) and hepatic damage resulting from local or systemic adverse effects of particular endotoxin, non-specific inflammatory reactions in response to intestinal perforations and cytotoxins generated and released by *Salmonella typhi* that have infected Kuffer cells (Sallie *et al.*, 1991). Treatment of infected animals with the different doses of the extract reversed the necrotic tissues gradually back to normal histo-architecture on day 16 especially with the high dose, indicating that the extract was effective at reversing these pathological changes in the liver. These effects could also be attributed to anti-bacterial activity of the extract against *Salmonella typhi*, which is in line with the report of Blessing and Uzoma (2014), who found *Milletia aboensis* to have exhibited significant antibacterial activity against clinical Isolates of *S. aureus*, *P. aeruginosa* and *K. Pneumonia*. This implies that, it is the elimination of the organism in the liver, that enabled the body to gradually metabolized and reduce the endotoxins and cytotoxins produced by the organism.

Conclusion

Ethanol root extract of *Millettia aboensis* reversed the hepatological changes caused by *S. typhi* infection in Wistar rats in our study.

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A Brief Author Biography

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