

Full Length Research Paper

***In vivo* and *in vitro* antibacterial activities of *Momordica charantia* on *Salmonella typhi* and its effect on liver function in typhoid-infected rats**

Adeyi, A. O.^{1*}, Jinadu, A. M.², Arojojoye, O. A.², Alao, O. O.², Ighodaro, O. M.² and Adeyi, O. E.³

¹Department of Zoology, University of Ibadan, Ibadan, Nigeria.

²Department of Biochemistry and Microbiology, Lead City University, Ibadan, Nigeria.

³Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria.

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Typhoid fever is a disease prevalent in the tropics. In spite of the availability of various therapies, treatment of patients with the disease has been quite challenging in the face of resistance to drugs used. *Momordica charantia* has been used locally to treat typhoid. This study investigated the antimicrobial potency of methanolic extract of *M. charantia* leaves on *Salmonella typhi* in male albino rats (Sprague dawley) and the effects of treatment on liver function. There were 5 groups of 10 rats each. 1 ml aliquot of the 4th dilution of *S. typhi* was administered orally to rats in four of the groups to be infected with typhoid, while the last group served as the control. Infected groups were thereafter treated with 100 and 200mg/kg of *M. charantia* and 10mg/kg of chloramphenicol, respectively for seven days, while the remaining group was not treated after infection. The effect of treatment on infection level, body weight and liver enzymes were thereafter investigated. Marked reduction in infection level was observed in all treated rats. Rats treated with 200 mg/kg of the plant extract had total clearance by the sixth day, while significantly lower ($p < 0.05$) infection level was recorded in rats treated with the plant extract than those treated with the standard drug. Mean body weight of all treated rat groups increased during treatment. Concentrations of total and direct bilirubin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) were higher ($p < 0.05$) in untreated rats than the treated rats. In conclusion, these results suggest that leaf extract of *M. charantia* is a potent antimicrobial drug against *S. typhi* with hepatoameliorative potentials.

Key words: Typhoid, *Momordica charantia*, liver, *Salmonella typhi*.

INTRODUCTION

Typhoid fever remains an important cause of illness globally with the annual incidence at 21 million cases of which 1 to 4% end fatally (Ivanoff, 1995). Most of the disease burden occurs in developing countries due to

poor sanitary conditions (Brown et al., 1996). The disease at its early stages is characterized by high fever, colic pain, anorexia, lethargy, malaise, dull continuous headache and diarrhea. At advanced stages there is

*Corresponding author. E-mail: delegenius@yahoo.com.

often a protracted fever and mental dullness, other symptoms may include intestinal bleeding, slight deafness and parotitis paratyphoid (Ackers, 200).

Typhoid is a systemic infection caused by salmonella enteric serotype typhi. This is a highly adapted human pathogen and possesses remarkable mechanism for persistence in host. *Salmonella typhi* is transmitted via the faecal-oral route, either directly from person to person or by ingestion of food or water contaminated with faeces (Ivanoff, 1995). The drugs for the treatment of typhoid fever are antibiotics such as ampicillin, chloramphenicol, trimethoprim, sulfamethoxazole and streptomycin, however, resistance to these drugs is now common (Threfall et al., 2001). In view of the increasing resistance to antibiotics and limited scope of vaccines, the need of the hour is to evaluate the efficacy of natural plant products for the treatment of this infectious disease. The usage of plant products as traditional health remedies is the most popular for about 80% of world's population and is reported to have minimal side effects (Grover and Yadav, 2004; Threfall et al., 1999; Threfall et al., 2001; Wain et al., 1997).

Bitter melon, also known as balsam pear is a tropical plant, widely cultivated in Asia, Africa and South America, and has been used extensively in folk medicine as remedy for eczema, hemorrhoids, scabies, skin conditions, infections, infestations of ticks and chiggers and stubborn sores and wounds, diabetes and enteric fevers (Girron et al., 1991). A decoction of leaf of bitter melon was also observed to inhibit the growth of *Bacillus subtilis*, *Salmonella* spp and *Escherichia coli* (Day et al., 1990). Bitter melon is composed of several compounds with confirmed multitherapeutic properties. In many Nigerian communities, traditional healers have recommended the use of leaf of *Momordica charantia* for the treatment of typhoid fever; however, there have been no scientific study on the efficacy of this plant for the treatment of the disease.

This study therefore evaluated the *in vitro* and *in vivo* antibacterial potency of *M. charantia* on *S. typhi* and also investigated the effects of the treatment on liver function in albino rats.

MATERIALS AND METHODS

Animals

Adult male albino rats of Wistar strain (Sprague dawley) weighing between 150 to 180 g were obtained from the animal house of the Department of Biological Sciences, University of Agriculture, Abeokuta for the study. They were kept in rat cages at room temperature ($27 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$) and a 12 h cycle of light and dark. They were given free access to rat pellet and water *ad libitum*. The experiment was performed in accordance with the National Institute of Health guidelines of care and use of laboratory animals.

Source of specimen and induction

S. typhi was obtained from the Microbiology laboratory of Nigeria Institute for Medical Research (NIMR) Lagos, Nigeria on already prepared *Salmonella shigella* Agar (SSA). The collected samples were immediately taken to the laboratory at Leadcity University, Ibadan, where they were incubated within 2 to 3 h of collection at 37°C for 24 h. Emerged colonies were stripped from the plate into normal saline. 1 ml aliquot of the 4th dilution of the sample was administered orally to the animals to induce typhoid. 1 ml of blood from the inoculated animals were drawn from the vein 24 h after and inoculated on prepared SSA on petri dishes. The plates were incubated at 37°C for 24 h. Emergence of colonies of *S. typhi* confirmed the induction of typhoid in the animals.

Plants, extracts preparation and treatment

Fresh leaves of *M. charantia* were collected from the campus of University of Agriculture, Abeokuta. The plants were authenticated at the Department of Forestry and Wildlife of the University of Agriculture, Abeokuta where the voucher specimen (MC20) was deposited. The leaves were air dried and blended using an electric blender. 200 g of the powdered leaves was soaked in one litre of absolute methanol and allowed to stand in the menstrum for 3 days. The extract was then filtered using Whatman filter paper No 1. The filtrate was poured into an evaporating dish and allowed to evaporate at room temperature to constant weight. The yield was about 4.6% w/w. Dilution to desired concentration was done when needed. Diluted extract was administered orally to the rats at 10:00 GMT for 6 days.

Experimental design

The animals were divided into 5 groups of 10 rats each.

1. Group A: This group served as the control. They were not treated throughout the experiment, but were given free access to normal animal pellet and water *ad libitum*.
2. Group M1: This group contained typhoid-induced rats treated with 100 mg/kg of *M. charantia*.
3. Group M2: This group contained typhoid-induced rats treated with 200 mg/kg of *M. charantia*.
4. Group C: This group contained typhoid-induced rats treated with 10 mg/kg of a standard antibiotic drug chloramphenicol.
5. Group U: This group contained typhoid-induced rats. They were not treated after typhoid induction but served as the positive control group.

The body weight of the animals was measured at pre and post treatment and recorded as mean weight per group.

Confirmation after treatment

1 ml of blood from the treated animals was drawn from the vein of treated animals 24 h after treatment each day and inoculated on already prepared SSA on petri dishes. The inoculated plates were incubated at 37°C for 24 h. The counts of emerged colonies were used to evaluate the efficacy of treatment.

Blood collection and dissection

At the end of the experiment, blood was collected from each rat by

cardiac puncture method. The blood was immediately transferred into appropriately labelled blood sample bottles containing anticoagulant.

In vitro sensitivity test using antibiotic disc

2.8 g of nutrient agar powder was weighed and added to 100 ml of sterile water. The mixture was sterilised (using the autoclave) at 121°C for 15 mins. The sterile nutrient agar was allowed to cool to 45°C and then poured into sterile petri-dishes containing the test organism. The petri-dishes were rotated anti-clockwise to allow even distribution of the agar and then left to solidify. Thereafter, a ring of disks of each (Mast Diagnostics, UK) containing single concentrations of each antimicrobial agent was then placed onto the inoculated surface. After 24 h incubation at 37°C, clear zones produced by antimicrobial inhibition of bacterial growth were measured in mm using a straight line ruler.

Estimation of liver function

Adopting the methods described by Tietz (1994), the levels of total and direct bilirubin, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined in the serum using assay kits from Roche Diagnostics on Roche modular (model P800) Mannheim, Germany.

Histological studies

The rat livers were collected and fixed in 10% formalin. The organs were processed routinely for histopathological evaluations.

Statistical analysis

Data obtained were expressed as mean \pm SEM. Significant difference between test and control groups was carried out using analysis of variance (ANOVA) of the statistical package for social sciences (SPSS) computer software, version 16.0 at 95% confidence intervals.

RESULTS

Colony counts of *S. Typhi* during treatment

Colony counts of *S. typhi* after induction ranged from 8.5 to 9.1 CFU (Table 1). Treatment of infected rats with the plant extract and chloramphenicol significantly decreased ($p < 0.05$) colony counts of *S. typhi* compared to the pretreatment values. Groups treated with the plant extract recorded significantly lower ($p < 0.05$) colony counts than rats treated with chloramphenicol, while rats treated with 200 mg/kg of the plant extract (M2) had total clearance by the 6th day of treatment.

Body weight of treated rats

The mean body weight of all typhoid-infected groups

reduced significantly ($p < 0.05$) after induction of typhoid (Figure 1). During treatment however, all treated groups recorded steady increase in body weight. Rats treated with the extract had higher weight gain than rats treated with chloramphenicol, while rats treated with 200 mg/kg of the extract also gained more weight than the group treated with 100 mg/kg.

In vitro sensitivity test of *S. typhi*

The efficacy of antibiotic sensitivity disc on *S. typhi* showed that ciprofloxacin had the highest zone of inhibition (16 mm) while cefuroxime, nitrofurantoin, tetracycline and ampicillin had the least (4 mm). However, methanolic extract of *M. charantia* had an inhibition zone of 14 mm (Table 2).

Liver function of treated typhoid rats

The concentrations of ALT and AST were highest in the untreated group (U) and lowest in groups M2 and C, respectively (Table 3). The concentrations of ALP and GGT were highest in groups C and A and lowest in groups U and C. Total and direct bilirubin were lowest in groups M2 and C and highest in group U.

Histology of the Liver

Liver of the control rats showed normal appearance of the hepatocytes with no visible lesion (Figure 2A). However, marked vacuolations of the hepatocytes was observed in liver of the untreated typhoid-infected rat (Figure 2D). The degree of degenerations were milder in the typhoid-infected group treated with 100 mg/kg of *M. charantia* while, rats treated with 200 mg/kg of the extract had normal hepatocytes with no visible lesion (Figure 2B and C).

DISCUSSION

The marked reduction in colony counts of *S. typhi* in blood of typhoid rats treated with various doses of *M. charantia* confirmed the antimicrobial potency of the plant and therefore suggests its efficacy in the treatment of typhoid fever. Result of this study also suggests that the plant is a stronger antimicrobial agent compared to chloramphenicol as rats treated with 200 mg/kg of the extract had total clearance of *S. typhi* by the 6th day of treatment, while rats treated with 100 mg/kg of the extract recorded lower colony counts than those treated with chloramphenicol at the doses tested. Increase in body

Table 1. Counts of *S. typhi* (10^4 cfu $^{-1}$ ml) in blood of typhoid-induced rats treated with *M. charantia*.

Group code	Confirmation Level	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
A	n.d	n.d	n.d	n.d	n.d	n.d	n.d
M1	8.6±2.3 ^a	7.5±3.8 ^{ab}	6.1±2.4 ^b	5.4±2.4 ^b	5.1±1.8 ^b	3.2±2.1 ^a	2.4±1.1 ^a
M2	9.1±2.5 ^{ab}	6.8±1.5 ^a	5.2±2.3 ^a	3.4±1.7 ^a	2.1±2.3 ^a	n.d	n.d
C	8.5±1.3 ^a	8.1±2.3 ^b	7.2±2.8 ^c	6.5±1.9 ^{bc}	6.1±1.4 ^c	4.2±2.5 ^b	3.7±1.5 ^b
U	8.8±1.5 ^a	8.5±2.1 ^{bc}	8.7±3.0 ^d	7.9±2.6 ^c	8.2±1.4 ^d	8.1±2.0 ^c	8.5±2.9 ^c

Values are mean ± SE. $n \leq 10$. Values within a column having different superscripts are significantly different at $p < 0.05$. n.d = not detected. A: Control rats; M1: Typhoid-infected rats treated with 100mg/kg methanol extract of *Momordica charantia*; M2: Typhoid-infected rats treated with 200mg/kg methanol extract of *Momordica charantia*; C: Typhoid-infected rats treated with 10 mg/kg Chloramphenicol; U: Untreated Typhoid-infected rats.

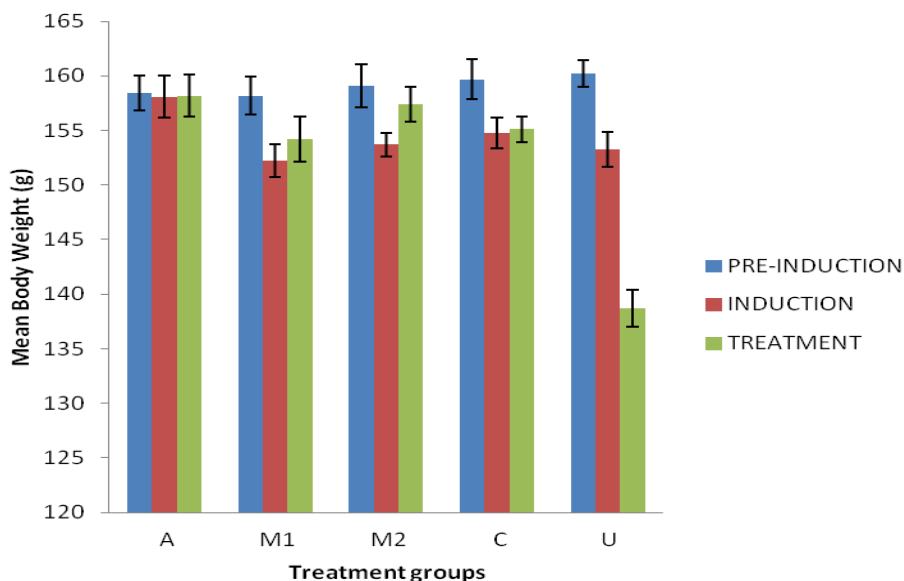


Figure 1. Body weight of treated typhoid-infected rats. A: Control rats; M1: Typhoid-infected rats treated with 100mg/kg methanol extract of *Momordica charantia*; M2: Typhoid-infected rats treated with 200mg/kg methanol extract of *Momordica charantia*; C: Typhoid-infected rats treated with 10mg/kg Chloramphenicol; U: Untreated Typhoid-infected rats.

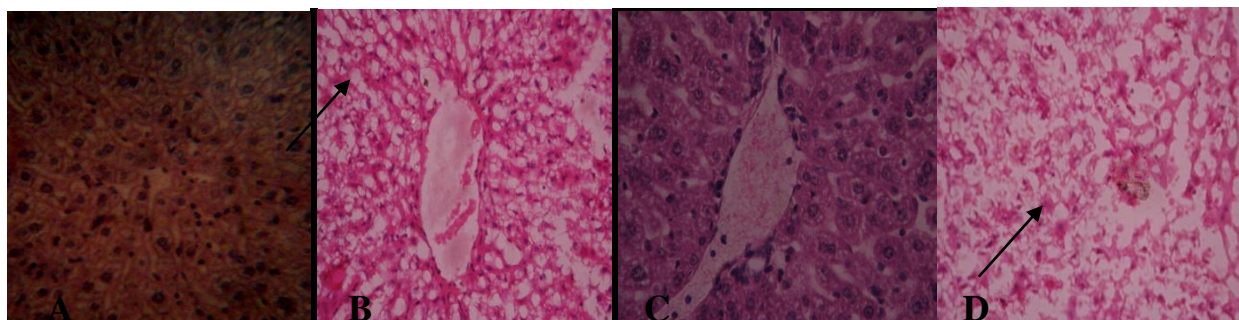


Figure 2. Histology of the liver. A. Liver of control rat show normal arrangement of the hepatocytes with no visible lesion. B. Liver of typhoid-infected rat treated with 100mg/kg of *M. charantia* show mild vacuolations of the hepatocytes (HP). C. Liver of typhoid-infected rat treated with 200mg/kg of *M. charantia* show normal hepatocytes. D. Liver of untreated typhoid-infected rat show markedly vacuolated hepatocytes. All panels were stained with H & E, magnification $\times 300$.

Table 2. Zones of inhibition of *Salmonella typhi* using antibiotic sensitivity disc.

Antibiotic disc	Diameter of zone of inhibition (mm)
Cefuroxime	4
Nitrofurantoin	4
Augmentin	7
Norfloxacin	11
Tetracycline	4
Gentamicin	9
Ciprofloxacin	16
Chloramphenicol	8
Ampicillin	4
Nalidixic Acid	5
<i>Momordica charantia</i>	14

Table 3. Concentration of liver enzymes of treated typhoid rats.

Group code	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Bilirubin (mmol/L)	
					Total	Direct
A	14.88±0.33 ^{ab}	29.14±0.45 ^b	13.80±0.43 ^{bc}	12.32±0.23 ^d	12.24±0.49 ^c	15.81±0.23 ^d
M1	14.30±0.32 ^a	29.47±0.55 ^b	11.04±0.13 ^a	10.65±0.32 ^b	11.83±0.16 ^b	12.69±0.43 ^c
M2	14.02±0.45 ^a	27.97±0.52 ^a	11.96±0.43 ^b	11.81±0.25 ^c	10.11±0.17 ^a	11.06±0.22 ^b
C	15.73±0.54 ^c	29.37±0.23 ^b	17.36±0.43 ^c	6.56±0.56 ^a	13.16±0.39 ^{cd}	7.87±0.48 ^a
U	16.71±0.12 ^d	30.94±0.24 ^c	19.32±0.22 ^d	18.49±0.24 ^e	15.85±0.26 ^d	20.07±0.26 ^e

Values are mean±SE. n ≤ 10. Values within a column having different superscripts are significantly different at p < 0.05. A: Control rats. M1: Typhoid-infected rats treated with 100mg/kg methanol extract of *Momordica charantia*; M2: Typhoid-infected rats treated with 200mg/kg methanol extract of *Momordica charantia*; C: Typhoid-infected rats treated with 10mg/kg Chloramphenicol; U: Untreated Typhoid-infected rats; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyltransferase.

weight observed in the rats during treatment also indicates the therapeutic potential of the plant as treated rats were observed to consume more food during treatment.

In vitro investigation of various antibiotics and the plant extract also confirmed a stronger antimicrobial potency of the extract on *S. typhi* than most of the antibiotics tested. Studies by Omoregbe (1996) had earlier reported that aqueous, ethanolic and methanolic extracts of *M. charantia* leaves presented antimicrobial potency against *E. coli*, *S. paratyphi*, *Shigella dysenteriae*, *Streptomyces griseus* and *Mycobacterium tuberculosis*. Fresh leaves extract of *M. charantia* had been reported to contain many secondary metabolites such as tannins, flavanoids and alkaloids which have been reported to have many biological activities including antimicrobial (Sankaranarayanan and Jolly, 1993).

The rise in serum levels of AST, ALT, ALP, GGT and total and direct bilirubin in serum of untreated rats can be attributed to the damage to the structural integrity of the liver cells, because these enzymes are cytoplasmic in

location and are released into the blood circulation upon cellular damages (Sallie et al., 1991). Histological observation of the liver of untreated rats revealed marked vacuolations of the hepatocytes which confirmed structural damage of the liver cells. Treatment of infected rats with the plant extract however, revealed gradual recovery of the injured liver cells, as all enzymes analysed were almost normal in the treated groups compared to the control and mild degeneration of the hepatocytes were also observed. This observation indicates the restorative effect of *M. charantia* on the liver cells.

The results of this study indicated that the methanol extract of the leaf of *M. charantia* contains potent antimicrobial agents(s) against *S. typhi* with restorative (and/or protective) influence against the biochemical and histological defects caused by the disease on the liver of the rats. Further studies are necessary to isolate and characterize the component of the leaf that is responsible for these observed effects and to elucidate its mechanism of action.

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