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SALMONELLA TYPHIMURIUM INFECTIONS TREATMENT IN MICE USING *MORINGA OLIFERA AQUEOUS* LEAVES EXTRACT

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ABSTRACT
Inhibitory activities of *Moringa olifera leaves* extract was investigated using mice. Mice were divided into 3 groups of 5 mice (n=15). Group **A** was neither infected nor treated with the extract and was used as control. Group **B** were orally challenged with 0.1 ml (3.0 x 10^4 CFU) of *Salmonella* Typhimurium and received no extract treatment and Group **C** mice were orally challenged with 0.1 ml (3.0 x 10^4 CFU) of *Salmonella* Typhimurium and were orally treated twice daily with 0.5 ml of extract containing 100 and 200 mg/kg body weight of the extract at 12 hours interval for six (6) days, respectively. The extract was found to be effective in the treatment of *Salmonella* infection as there was a significant association between the extract treatment and increase in Body weight and Food consumption in the infected and treated group (C). An indicative that *Moringa olifera* leaves contain bioactive compounds which could be a source of new drug for the treatment of diseases cause by Salmonella Typhimurium.

KEYWORDS: *Moringa olifera leaves, Salmonella* Typhimurium.

INTRODUCTION
Today, most of the world population depends upon plant based drugs for their primary health care needs (Ahmed *et al.*, 2008). Plant based medicines are now considered as an alternative approach to treat various diseases because it is considered to be safer and having no side effect due to the presence of natural ingredient (Bagio *et al.*, 2008). Salmonella Typhimurim is a bacterium that causes Paratyphoid fever with a fatality rate of 10% (Alawode, 2003). The disease is a cause of concern and a major public health problem in developing countries (Asia, Africa) especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Anita *et al.*, 2002). Laboratory mice have served as an important animal model for research in medicine (Krinke and George, 2000). Many of the existing drugs are known to cause various side effects; therefore evaluation of the potency of *Moringa olifera* in mice will provide an alternative source of treatment. The goal is to find whether the *Moringa olifera* leaves extract has an antibacterial activity on *Samonella* Typhimurium in mice.

MATERIALS AND METHODS
Collection of plant and Preparation of plant extract
Fresh leaves of mature *Moringa olifera L.* were collected and were identified in the department of Biological Sciences Kebbi State University of Science and Technology Aliero with Voucher number *L.*75. The leaves were gently cleaned and washed under running tap water to remove dirt after which they were air-dried at room temperature in the absence of sunlight for two weeks and then ground into powdered form using clean laboratory motor and pestle, the powder was stored in a sterile plastic container (Muktar and Tukur 1999). 100g of the powder (*Moringa olifera* leaves) was soaked in 500ml of 95% ethanol in a conical flask for two weeks with regular shaking at room temperature. This was then filtered and the solvent evaporated using rotary evaporator and kept at 4^0C before used (Alade and Irobi 1993).

Phytochemical Screening
The crude plant extract obtained was subjected to preliminary phytochemical screening to identify the chemical constituents. The methods of analysis employed were those described by (Sofowora, 1993).

Source of mice used in the study
Mice used in this study were aged 3 months and weighing 200–250g. The mice were obtained from Animal House of the Usman Danfodiyo University Sokoto Nigeria. The mice were assigned randomly and individually in micro-isolated cages in the same room on a 12hr light-dark cycle. The mice were allowed to acclimatize to their new environment for 2weeks before

inoculation and were tested for one-week period to ensure that they were negative for *Salmonella* Typhimurium. Sterile food and deionized water were provided from the day the mice were procured until the completion of the experiment (Kuramoto and Takashi, 2012).

Identification of test organism

The preliminary identified isolates by biochemical tests as Salmonellae were further subjected to serological test according to Kauffmann-White scheme using slide agglutination test. The polyvalent Somatic (O) and Flagellar (H) Salmonella anti-sera used were obtained from Staten's Serum Institut (SSI), Copenhagen, Denmark (Bagudo *et al*., 2014).

Infection and treatment of mice

A loopful of the organism stored in nutrient agar slant was transferred into test tube containing 10mls of sterilized peptone water and incubated at 37^{O}C for 24hrs. Mice were divided into 3 groups of 5 mice (n=15). Group **A** was neither infected nor treated with the extract and was used as control except that the animals were given equal volume of distilled water. Group **B** were orally challenged with 0.1 ml (3.0 x 10^4 CFU) of *Salmonella* Typhimurium and received no extract treatment and Group **C** mice were orally challenged with 0.1 ml (3.0 x 10^4 CFU) of *Salmonella* Typhimurium and were orally treated twice daily with 0.5 ml of extract containing 100 and 200 mg/kg body weight of the extract at 12 hours interval for six (6) days, respectively (Sumitra *et al.*, 2011). All animals were allowed to freely access sterile food and distilled water throughout the experiment (Muktar and Tukur, 2009).

Detection of Salmonellae from Mice faeces

Stool samples were collected from anal swab of the mice and were serially diluted and were then cultured on Salmonella-Shigella agar for Salmonella detection and number of colonies appearing were counted using colony counter (Bagudo *et al*., 2014).

Measurement of body temperature

Body temperature in degree Celsius (^{o}C) of the mice were taken daily to check for any change in body temperature due to infection for the whole period of the study and for the whole mice in three groups by inserting clinical thermometer in to the anus of the mice for ten minutes (Zampini *et al*., 2009).

Measurement of food consumption of the rats

Food consumption by the mice was measured by measuring the amount of food in grams given to each mice in its micro cage per day and subtracting the amount of food remaining in the next day (Tan *et al*., 1991).

Table 1 Phytochemical analysis Results

Phytochemicals	Ethanol extract
Flavonoids	+
Saponins	+ +
Tannins	-
Steroid	+++
Terpenoids	++
Anthraquinones	++

KEY: - = absent, + = slightly present, **++** = moderately present, +++ = highly present.

RESULTS

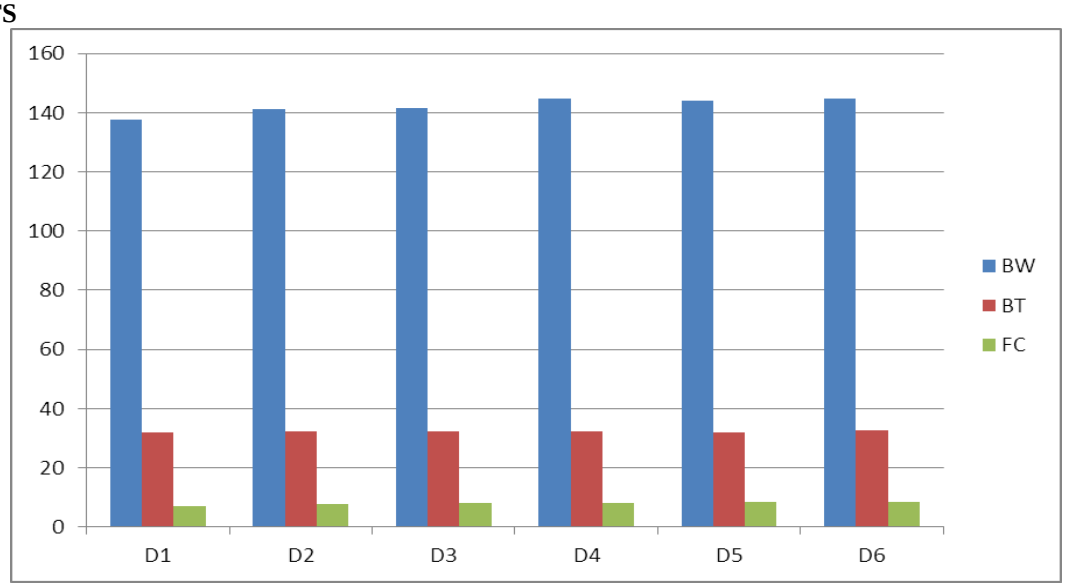

Chart 1: Showing the average distribution of Body weight, Body temperature and food consumption of the rats in the control group (A) (X-axis represents number of days and Y-axis represents BW, BT and FC).

KEY: BW= body weight**, BT**= body temperature**, FC**= food consumption**, INT**= infected not treated**, IWT**= infected with treatment**, D**= days.

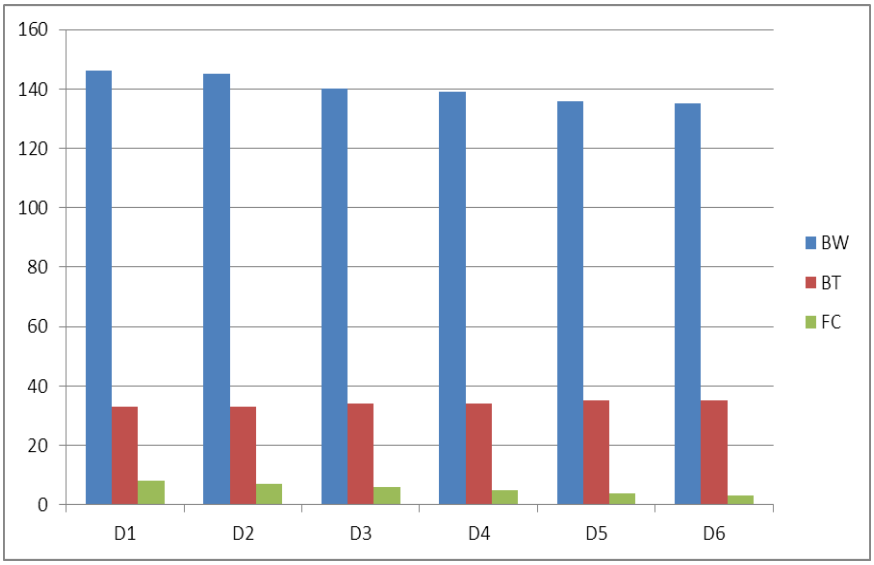

Chart 2: Showing the average distribution of body weight, body temperature, food consumption of the rats in the infected not treated group. (X-axis represents number of days and Y-axis represents BW, BT and FC).
KEY: BW= body weight**, BT**= body temperature**, FC**= food consumption**, INT**= infected not treated**, IWT**= infected with treatment**, D**= days

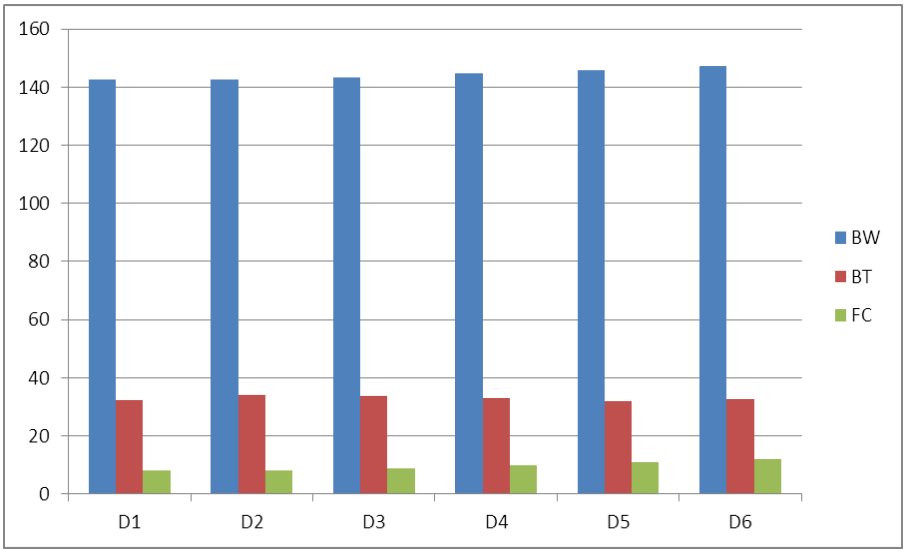

Chart 3: Showing the average distribution of body weight, body temperature, and food consumption of the rats in the infected with treatment group. (X-axis represents number of days and Y-axis represents BW, BT and FC).
KEY: BW= body weight**, BT**= body temperature**, FC**= food consumption**, INT**= infected not treated**, IWT**= infected with treatment**, D**= day

Day	Control	infected not treated	Infected and treated
			0
D1	Nil	0	$9.8X10^5$
D2	Nil	$7.2X10^5$	$9.3X10^5$
D3	Nil	$9.9X10^5$	$6.6X10^5$
D4	Nil	$1.21X10^5$	$2.8X10^5$
D5	Nil	$1.34X10^5$	$1.3 X10^5$
D6	Nil	$1.72X10^5$	

DISCUSSION

All the mice were found negative for *Salmonella Typhiminum* in faeces before inoculation. Mice in group B (infected without treatment) and group C (infected with treatment) shed Salmonellae in faeces after inoculation, while group A remained negative as shown in Chart 1. Number of *S.* Typhimurium shed in faeces of the mice decrease in Group C after treatment with plant extract from $7.2x10^5$--$9.1X10^5$. The number increased in group B (infected without treatment) from $8.1x10^5$ to $9.9x10^5$ and group A control shed negative organism in faeces in day three. The result revealed that there was a lot of variation in the percentage of mice that shed organism in the experiment. The organism shed in faeces increases in group B infected without treatment $8.1x10^5$-$1.89x10^5$ and decreased in Group C infected with treatment from $9.4x10^5$-$1.2x10^5$. There was a significant association between treatment and time ($P<0.05$) over the course of study. However, when comparing treatment groups at specific sampling days the proportion of mice shedding faecal *Salmonella* in group B (infected without treatment) was significantly higher ($P< 0.05$) than group C (infected with treatment). Group A mice maintained significant increase in food consumption, body weight and slightly normal body temperature while mice in group B showed decrease in food consumption, body weight and increase in body temperature as the number of study days increases and mice in group C at the begging in of the study showed decrease in food consumption, body weight but letter increase as the extract treatment continues

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