



## Curative potential of *Gbogbonise Epa Ijebu* herbal remedy in male Wistar rats infected with *Salmonella enterica* serovar typhi

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### ABSTRACT

**Background & Aim:** Commercially sold herbal remedies are fast gaining recognition among the Nigerian populace for the treatment of various ailments. The aim of this study was to assess the curative potential of *Gbogbonise Epa Ijebu* herbal remedy in male Wistar rats infected with *Salmonella enteric* serovar *typhi* (*S.typhi*).

**Experimental:** A total of 60 male Wistar rats weighing 150±50 g (mean±SD) were randomly assigned into 10 groups of 6 rats each. Group 1, 2, 3, 7, 8, 9 and 10 were infected with one ml of *S. typhi* suspension with a concentration of 10<sup>6</sup> CFU/mL in phosphate buffered saline (PBS). Group 4, 5 and 6 which served as the Zero control, undiluted herbal control and diluted herbal control, respectively. Group 7 and 8 were treated with 500 mg/Kg/bid and 250 mg/kg/bid herbal remedy, respectively, while group 9 and 10 were treated with equal volume of Ciprofloxacin and herbal remedy, 500 mg/kg/bid and 250 mg/kg/bid, respectively, orally twice daily for 7days. Bacterial loads in rectal swab, intestine and gall bladder, as well as the anti-*S.typhi* O and H serum antibodies of the test and control rats were evaluated at pre-infection, post-infection and post-treatment phases using standard microbiological and serological methods.

**Results:** The outcome of this study showed that the administration of the herbal remedy (500 mg/Kg/bid) resulted in a significant reduction ( $P<0.001$ ) in the rectal (1.8±0.4 Log CFU/mL), intestinal (4.25±0.05 Log CFU/Intestine) and gall-bladder (1.85±0.25 Log CFU/Gall-Bladder) bacterial load, as well as the anti-*S. typhi* O (93.3±22.3) and H (93.3±22.3) serum antibodies level of the test groups compared to the infection control (3.8±0.20 Log CFU/mL, 8.3±0.10 Log CFU/Intestine, 4.3±0.06 Log CFU/Gall-Bladder and 173.3±32.1, respectively).

**Recommended applications/industries:** The outcome of this study further underscores the curative potential of the herbal remedy as claimed by the manufacturers and vendors.

### 1. Introduction

Herbs are plants or parts of plants valued for their medicinal, aromatic, or savory qualities (Alwhaibi et

al., 2017). Plants are being used as the backbone for medical treatments for much of human history, and this

type of traditional medicine is still used today (Vicker, 2007). Many plant-derived substances are used as the basis for evidence-based pharmacological medications in modern medicine. They can be derived from any part of the plant, but leaves, roots, bark seeds, and flowers are the most frequently used. They can be consumed, ingested, drunk, inhaled, or administered topically to the skin (WHO, 2008; Ileoma *et al.*, 2021; Enitan *et al.*, 2022).

Despite the fact that the use of phytotherapy is on the rise, consumers and health professionals sometimes overlook critical issues such as dangerous drug-herb combinations, side effects, and the ineffectiveness of many treatments (Rahman and Al-Rashid, 2019). Only herbal medicines made in accordance with the logical phytotherapy principle are of acceptable quality, ensuring their safety and efficacy. Herbal medicine phytopreparations should meet all standard requirements to the greatest extent possible (Rahman and Rashid, 2019; Jeremic *et al.*, 2019).

Herbal medications can be obtained from a variety of places, including road sides, pharmacies, health food stores and websites, so a thorough assessment of their safety and effectiveness is critical, especially given the current increase in the rise of antibiotic resistant pathogens (Ben *et al.*, 2019). The society at large has been encouraged to use herbal remedies for curative purposes and there has been a major increase in the use of herbal remedies in African countries. In Nigeria for instance, the use of herbal remedy is vastly used to treat ailments like malaria, typhoid, fever, pile, diarrhea, and constipation. Examples of such herbal remedies include: Eversure, Blessed Mother, Goko Cleanser, Ezinco Staphylococcus wiperand *Gbogbonise Epa Ijebu* 200 cure (Enitan *et al.*, 2022).

*Gbogbonise Epa Ijebu* is one of the common commercially sold herbal remedy in Ogun State, South-West Nigeria. Juice of *Citrus aurantifolia*, *Citrus aurantium*, and fruits of *Aframonium melegueta* (alligator pepper), as well as animal parts including a type of rat called *Rattus norvegium*, snake heads of various kinds and scorpions are all used in the preparation of the concoction according to information gathered from local herbalists. These various ingredients are well documented for their medicinal benefits including antimicrobial activities (Newman, 2001; Doherty *et al.*, 2009; Madhuri *et al.*, 2014; Mukherjee *et al.*, 2017; Abubakar *et al.*, 2018, Martins *et al.*, 2021). The animal parts are dried, grounded to

powder, combined with the plant parts and boiled in a sizable pot. After cooling, the concoction is dispensed into small bottles and labeled for sale. Pap (slurry of milled corn produced in boiling water), is mixed with small amounts of the paste and consumed. According to the vendors, the wonder drug cures a variety of illnesses including typhoid fever caused by *S. typhi* (Adeleye *et al.*, 2008; Belonwu *et al.*, 2013).

Previously, Ileoma *et al.* (2021) reported the effects of *Gbogbonise Epa Ijebu* herbal remedy on some haematological parameters in male rats infected with *S. typhi*. And in another recent study, Enitan *et al.* (2022) reported on the microbiological quality and efficacy of on some uropathogens of the same herbal remedy. Many of the pharmacological properties associated with *Gbogbonise Epa Ijebu* are believed to be directly or indirectly attributable to the presence of some important phytochemicals including Flavonoids, alkaloids, saponins, anthraquinones, tannins, and cardiac glycosides (Adeleye *et al.*, 2009; Enitan *et al.*, 2022).

*S. typhi* is a Gram-negative, flagellated bacterium that is responsible for typhoid fever and has been a burden on developing nations for generations (Shu-Kee *et al.*, 2015; Gut *et al.*, 2018). Serologically, the bacterium is positive for the lipopolysaccharide antigens O9 and O12, as well as the polysaccharide capsular antigen vi (Parry, 2002). In contrast to Vi positive strains, antigen Vi-negative bacteria appear to be less contagious and virulent (Crump, 2015). *S. typhi* causes a life threatening disease called enteric fever or typhoid fever which is endemic in Nigeria with varied morbidity and mortality rates (Cheesebrough, 2006). The global burden of typhoid fever is estimated to be over 26.9 million people, and Nigeria is not immune to the disease and its effects. Antibiotics seem to be less successful in treating bacterial infections such as typhoid fever due to the alarming growth of antibiotic-resistant Salmonella strains, the impact of antibiotics on normal gut microflora, and antibiotic-associated diarrhea (Ramirez *et al.*, 2020).

Currently, research reports have provided concrete evidence that microorganisms are developing resistance to available antibiotics (Ben *et al.*, 2019; Amarasiri *et al.*, 2022), thus leading to the inability to treat certain infections, therefore such concerns have motivated the interest in the application of alternative, novel, non-antibiotic based method for preventing and treating *S. typhi* infection and as a results of advocacy and

continuous search for alternative medicine and herbal remedies is being intensified. The aim of this study is to assess the curative potential of a commercially sold herbal remedy (*Gbogbonise Epa Ijebu 200 Cure*) in adult male wistar rats infected with *S. typhi*.

## 2. Materials and Methods

### 2.1. Study design

This is an experimental-laboratory-based study using animal model. The study lasted for a period of 2 months (September-November 2020).

### 2.2. Study setting

The survey was performed at Babcock University's Experimental Animal House in Ilishan-Remo, Ogun State, a Seventh-day Adventist institution of higher learning. Ilishan-Remo community is a geopolitical ward in Ikenne Local Government Section of Ogun State, located in the tropical area of Nigeria's south-western region at 7°29'00"N 2°53'00"E. Trading and farming are the most prevalent jobs among the residents, and they are known for growing rubber, cocoa, cashew nuts, plantains, and other agricultural items. Despite the availability of effective medical services, the local population continues to rely on plants as medicines for both curative and prophylactic purposes.

### 2.4. Test herbal product

Samples of commercially sold herbal remedy, "*Gbogbonise Epa Ijebu 200 Cure*" was procured from local vendors in Ilishan-Remo Community of Ogun State. Each bottle contained 100 mL of the herbal paste (Figure 1). According to the manufacturer, *Gbogbonise Epa Ijebu 200 Cure* herbal remedy can be used to treat ailments such as stomach ache, headache, rheumatism, sores, cuts, snake bites, scorpion bites, cough, piles, chest pain, convulsion etc.

### 2.5. Preparation of herbal product

The herbal product was brought out from the refrigerator, allowed to defrost to room temperature and then properly shaken before use. The herbal remedy paste was reconstituted using sterile distilled. Five grams (5g) of the herbal remedy paste was dissolved in 10 mL of sterile distilled water to obtain an herbal remedy solution with a concentration of 500 mg/mL, while a lower concentration (250 mg/mL) was

prepared by making a 1:2 dilutions (*i.e.*, 5 mL of 500 mg/mL herbal solution + 5 mL of sterile distilled water). All these were done aseptically. The solutions were kept in the refrigerator until use.

### 2.6. Antibiotic

Ciprofloxacin® (Geltec Pvt Ltd, India) was obtained from the Pharmacy Unit, Babcock University Teaching Hospital, Ilishan-Remo, Ogun State. Every day, 20 mL of Ciprofloxacin® suspension was made by dissolving twenty (20) tablets of Ciprofloxacin® (500mg each) in 20 mL of sterile distilled water to prepare a 500 mg/mL concentration solution.



Figure 1. *Gbogbonise Epa Ijebu 200 Cure*

### 2.7. Experimental animals

A total of 60 male albino rats weighing 150±50g (mean±SD) were obtained from the small animal house, University of Ibadan (Oyo State, Nigeria) and were clinically inspected upon arrival for any signs of abnormality or disease. The animals were housed separately in well ventilated wire-bottom steel cages under hygienic conditions at 25±2°C and a relative humidity of 45–50% at the Experimental Animal House, Department of Animal Science, School of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo (Ogun State, Nigeria). The rats were randomly divided into groups of 10 consisting of 6 rats each and were provided and fed with standard rat diet (10g/100g body weight) twice daily and tap water *ad libitum*. For a period of 14 days prior to the start of the study, the rats were allowed to settle in the Animal House with a regular 12-hour light-dark cycle. All animal experimental experiments were carried out in compliance with the Institute for Laboratory Animal Research's Current Animal Care Regulations and Standards (ILAR, 1996).

### 2.8. Pre-infection phase

To assay for any pre-existing infection with *S. typhi*, about 0.5g of fecal sample aseptically collected from each rat was inoculated onto MacConkey agar (MCA)

and sub-cultured onto Salmonella-Shigella agar (SSA) medium partly selective for Salmonella. Colonies were sub-cultured onto Nutrient agar plates and incubated at 37°C for 18-24 hours from overnight cultures of viable colonies on selective media. Colony morphology, Gram staining features, motility, and biochemical testing were used to identify isolates as described by Cheesbrough (2006). Bergey's Manual for Determinative Bacteriology was used to identify the isolates (Buchanan and Gibbons, 1974). For the investigation, only rats with a negative stool bacteriological culture for *S. typhi* were used.

## 2.9. Infection phase

### 2.9.1. Test isolate

Stock culture of *S. typhi* from patients with laboratory confirmed typhoid fever was obtained from the Microbiology Laboratory of the Olabisi Onabanjo University Teaching Hospital (OOUTH), Shagamu, Ogun State.

### 2.9.2. Preparation of test isolate

The isolate was re-identified biochemically using standard operating procedures after being sub-cultured onto selective and differential solid media from preserved agar slant as described by Cheesbrough (2006).

### 2.9.3. Standardization of inoculum

Five colonies from the isolate's pure culture were introduced into nutrient broth (NB) and incubated for 18 to 24 hours at 37°C. The surface viable count was performed according to Miles and Misra (1938). The bacteria were first rinsed in sterile phosphate buffered saline (PBS) before being adjusted to the desired concentration. Turbidity of the bacterial suspension (*i.e.* overnight nutrient broth with population density of  $10^7$  CFU/mL), was adjusted to match that of 0.5 McFarland standard ( $10^6$  CFU/mL) by making a dilution of 1:10 in sterile nutrient broth.

### 2.9.4. Induction of experimental typhoid fever

The typhoid fever model used by Nwankpa *et al.* (2014) was adapted for this study. Briefly, following overnight fasting, one mL of *S. typhi* suspension in phosphate buffered saline at a concentration of  $10^6$  CFU/mL (PBS) were administered orally to the rats using intragastric tube to induce typhoid (Kirby Bauer, 1960) in Group 1, 2, 3, 7, 8, 9 and 10 except, the zero

control, undiluted herbal control, and diluted herbal control which were assigned to groups 4, 5, and 6. Animals were observed daily for seven days for clinical signs and symptoms consistent with typhoid fever (e.g., rise in body temperature, anorexia, diarrhea etc) were recorded.

## 2.10. Post-infection phase

Successful induction of typhoid fever was confirmed using both serological and culture methods before intervention with herbal remedy commences. Rectal swabs, stool and serum samples were collected from each inoculated rat 7 days' post-infection. These samples were treated and handled as infectious using standard precautions (WHO, 2019).

### 2.10.1. Widal test

The Widal test is a serological technique which detects the presence and levels of agglutinating antibodies against the somatic (O) and flagellar (H) antigens in serum using standard *S. typhi* O and H antigen suspensions.

#### 2.10.1.1. Qualitative Widal test

Qualitative widal test is used to test the presence of agglutinating antibodies against the *S. typhi*-somatic (O) and flagellar (H) antigens in serum. Briefly, a drop of the test serum was placed on a clean grease-free slide and 1-2 drops of the standard *S. typhi*-antigen suspension was then added. The serum-antigen suspension was mixed evenly using an application stick. The mixture was rocked for about 5 minutes and observed for agglutination, *i.e.* visible clumping.

#### Probable result

Agglutination is a positive test that indicates the rat's serum has antibodies to the 'O' and 'H' antigens, confirming typhoid fever infection. The absence of agglutination is a negative test that indicates the rat does not have typhoid fever (Cheesbrough, 2006; Youssef *et al.*, 2010).

#### 2.10.1.2. Quantitative Widal test

The quantitative widal test is used to determine the levels of agglutinating antibodies against the *S. typhi* O (somatic) and H (flagellar) antigens in serum. Briefly, two set of 12 test tubes properly labeled were arranged in wooden racks to determine the serum level of agglutinating antibodies against the *S. typhi*-O

(somatic) and H (flagellar) antigens in infected rats. Using the doubling dilution approach, the antibody level was determined by diluting the serum. (*i.e.*, 1 in 20, 1 in 40, 1 in 80, 1 in 160, etc.). Afterwards, standard *S. typhi*-O and H antigen suspension was put on each set of test tubes. The tubes were then incubated at 37° for 1 hr in a hot water bath (during which time the bacterial cells settle) along with a control tube containing only the antigen suspension to check for auto agglutination of the reagent. The tubes were examined for agglutination. The titration test ends when the tube in each row with the highest dilution shows signs of agglutination.

### **Probable result**

The titer is defined as the reciprocal of the serum dilution at this end-point. Values greater than or equal to ( $\geq$ ) 160 is considered positive.

### **2.10.2. Rectal swab and tissue homogenate culture**

The rectal swab specimen collected from each rat was streaked separately on *Salmonella-Shigella* Agar (SSA) plate. The inoculum was incubated at 37°C for 18-24 hours. Any pale colored colony (suggestive of non-lactose fermenter) was sub-cultured on Nutrient agar, MacConkey agar and Triple Sugar Iron (TSI) Agar. Following another 18-24 hours of incubation, bacterial growth was subjected to Gram staining, motility, and biochemical assays to confirm the organism, as described previously by Cheesbrough (2006), Francis *et al.* (2008) and Mokhtari *et al.* (2015). Serotyping of the *S. typhi* isolates was performed using *Salmonella* O, H and Vi antisera supplied by BSL Global. To determine the bacteria burden in the intestines and gall bladder of the infected rats, two rats from each infected group were sacrificed by cervical dislocation as described by Ochei and Kolhatkar (2006). The intestines were removed and ripped open under sterile conditions. On *Salmonella-Shigella* agar (SSA) plates, the open surface of each intestine was imprinted. The organs were rinsed with 100 mL of sterile physiological saline solution, followed by sterile filter paper absorption of the saline solution sticking to the organ tissue. Each intestine was homogenized in 5

mL of distilled water. The colonies were counted after serial 10-fold dilutions of the tissue homogenates were inoculated onto agar (0.1 mL/9-cm diameter plates) and incubated overnight at 37°C. The number of colony-forming units (CFU) per milliliter of faeces and intestine were used to calculate bacterial counts.

### **2.11. Treatment phase**

The herbal remedy was assessed for its curative ability to eliminate *S. typhi* from the gastrointestinal tract of rats. Therapy was commenced 7 days' post-bacterial challenge and elapsed on the 7<sup>th</sup> day. Experimental animals were treated as shown in Table 1. Group 1, 2 and 3 served as the infection control (no treatment), positive control (received 500 mg/kg/bid of Ciprofloxacin) and negative control (received sterile phosphate buffer saline), respectively. While Group 4 served as the Zero control (no infection and no treatment). Uninfected rats in Group 5 and 6 received undiluted and diluted herbal remedy suspension, respectively. While, infected rats in Group 7 and 8 received undiluted and diluted herbal remedy suspension, respectively. Infected rats in Group 9 received equal mixture (50:50) of undiluted herbal remedy suspension and Ciprofloxacin (500 mg/kg/bid), while Group 10 received equal mixture (50:50) of 1:2 diluted herbal remedy suspension and Ciprofloxacin (500 mg/kg/bid) post-infection. The volume of suspension (5 mL/Kg body weight) to be administered orally to each single rat making use of intragastric tube was calculated and recorded prior to the start of the treatment phase. Following that, the rats were monitored on a daily basis for any changes. For 7 consecutive days, study animals were given a suitable volume of suspension (5 mL/kg), shaken gently prior to administration and delivered by oral gavage twice daily, every 12 hours (between the hours of 6.00-6.30 AM/PM). Microbiological and serological parameters were used to evaluate treatment response. At day 8 and 16, two rats from each group were sacrificed and the total CFU recovered from rectal swab, stool, whole-intestine and gall bladder homogenates were determined. Serum antibody titer was also measured.



**Table 1.** Experimental pharmacological protocol

Groups	n	Treatments
G1	6	Infected and received no treatment (Infection Control )
G2	6	Infected and received 500 mg/kg/bid of Ciprofloxacin (positive control)
G3	6	Infected and received sterile PBS (Negative control)
G4	6	Uninfected and received no treatment (Zero control)
G5	6	Uninfected and received 500 mg/kg/bid herbal remedy
G6	6	Uninfected and received 250 mg/kg/bid herbal remedy
G7	6	Infected and received 500 mg/kg/bid herbal remedy
G8	6	Infected and received 250 mg/kg/bid herbal remedy
G9	6	Infected and received equal mixture (50:50) of 500 mg/kg/bid herbal remedy and 500 mg/kg/bid+ Ciprofloxacin
G10	6	Infected and received equal mixture (50:50) of 250 mg/kg/bid herbal remedy and 500 mg/kg/bid+ Ciprofloxacin

n = Number of rats per group.

### 2.12. Weight measurement of experimental animals

The animal's body weight (control and treated groups) were measured at pre-infection phase, infection phase, treatment phase and recovery phase with the use of electronic sensitive analytical balance.

### 2.13. Specimen collection from experimental animals

#### 2.13.1. Blood collection

Prior to terminal sacrifice of the experimental animals, blood specimen for serological analysis was collected from the tail of each rat. Briefly, the back of each rat was placed on a properly disinfected cork board and strapped with two adhesive tapes across the forelegs and hind legs. The posterior end of each animal's tail was held still with a forceps and aseptically cut open using a sterile surgical scissor. As the animal bleeds from the tail, the dry cotton wool was used to clean the first drop of blood and subsequent drops were collected into a plain bottle until the bleeding stops and the cut site pressed with a piece of dry cotton wool. To collect a cardiac blood specimen, a 40 by 0.8 mm needle was inserted at the center line towards the sternum and pushed forward at an angle of 45° till it punctured the heart. After then, the needle was advanced until blood began to flow into the syringe. About 5 mL of cardiac blood specimen was collected, thereafter the needle was then withdrawn and the blood specimen collected was transferred into a plain bottle. The blood samples were taken to the laboratory and allowed to coagulate for roughly an hour before being retracted and centrifuged at 3,500 rpm for 10 minutes at room temperature. The serum was then transferred to a new sterile plain bottle. The yielded serum was transferred to another clean sterile plain bottle and was used for the detection and titration of serum anti-*S. typhi* antibodies.

#### 2.13.2. Rectal swab collection

Briefly, latex free gloves were worn before starting the rectal swab collection procedure to avoid self-contamination. A sterile rectal swab stick 1-2 inches was carefully inserted into the rectum of each rat to swab the rectal walls (lateral, anterior and posterior fornices). The swab stick was kept in the rectum for 20 seconds before removing it in an aseptic manner and corked properly immediately.

#### 2.13.3. Organ harvest

Briefly, according to Ochei and Kolhatkar (2006), the animals were sacrificed through cervical dislocation. The peritoneum was cut open using sterile surgical knives and forceps in an aseptic manner. The intestine and gallbladder of each animal was harvested under sterile conditions and cut open. The organs were rinsed with 100 mL of sterile physiological saline solution, followed by sterile filter paper absorption of any remaining saline solution adhering to the organ tissue. Each organ was homogenized in 5 mL of distilled water for the purpose of bacterial load counts.

### 2.14. Ethical approval

The Babcock University Health Research Ethics Committee (BUHREC) granted ethical permission for the study, with the registration number BUHREC 216/20.

### 2.15. Data analyses

The antibody titer, as well as the bacterial colony forming unit discovered in the rectal swab, intestine, and gall bladder specimens were presented as means of two rats using histograms. Using the SPSS-18.0 (Statistical programs for social Scientists, version 18.0) statistical tool, data were analyzed using one-way analysis of variance (ANOVA) and Tukey-Kramer

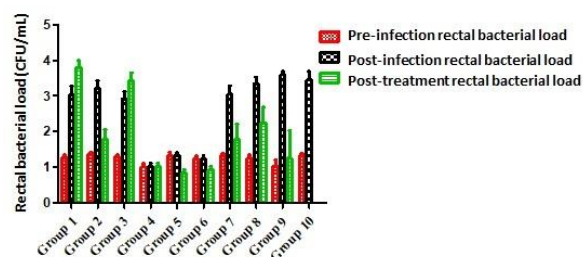
Multiple Comparisons Test. P values less than 0.05 were deemed significant.

### 3. Results and discussion

This current study evaluated the curative potential of *Gbogbonise Epa Ijebu* herbal remedy in male Wistar rats infected with *S. typhi*.

#### 3.1. Effect on rectal bacterial load

The rectal bacterial load of 500 mg/kg/bid herbal remedy treated rats (Group 7:  $1.8 \pm 0.41$  Log CFU/mL) and 500 mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 9:  $1.25 \pm 0.79$  Log CFU/mL) decreased significantly ( $P < 0.001$ ) when compared with the infection control group ( $3.8 \pm 0.20$  Log CFU/mL) at post-treatment. Also, this parameter in 250 mg/kg/bid herbal remedy treated rats (Group 8:  $2.25 \pm 0.44$  Log CFU/mL) and 250 mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 10: 0 Log CFU/mL) decreased significantly ( $P < 0.001$ ) when compared with the infection control group ( $3.8 \pm 0.20$  Log CFU/mL). There were no significant differences ( $P > 0.05$ ) in the rectal bacterial load of the rats when 500 mg/kg/bid and 250mg/kg/bid herbal remedy were tested singly at post-treatment ( $1.8 \pm 0.41$  Log CFU/mL and  $2.25 \pm 0.44$  Log CFU/mL, respectively) and in combination with Ciprofloxacin ( $1.25 \pm 0.79$  Log CFU/mL and 0 Log CFU/mL, respectively) (Figure 2).



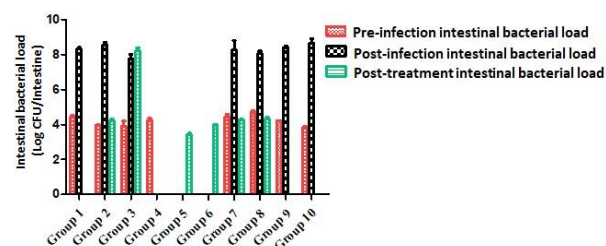
**Figure 2.** A histogram showing the rectal bacterial load (CFU/mL) of control and test rats at different intervention phases.

**Keys:** Group 1= Infection Control, Group 2= Positive control, Group 3= Negative control, Group 4= Zero control, Group 5 = Uninfected and received 500 mg/kg/bid herbal remedy, Group 6= Uninfected and received 250 mg/kg/bid herbal remedy, Group 7= Infected and received 500 mg/kg/bid herbal remedy, Group 8= Infected and received 250 mg/kg/bid herbal remedy, Group 9= Infected and received equal mixture (50:50) of 500 mg/kg/bid herbal remedy and 500 mg/kg/bid+ Ciprofloxacin, Group 10= Infected and received equal mixture (50:50) of 250 mg/kg/bid

herbal remedy and 500 mg/kg/bid+ Ciprofloxacin, CFU= Colony forming unit.

#### 3.2. Effect on intestinal bacterial load

The intestinal bacterial load of 500mg/kg/bid herbal remedy treated rats (Group 7:  $4.25 \pm 0.05$ ) and 500mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 9: 0 Log CFU/Intestine) decreased significantly ( $P < 0.001$ ) when compared with the infection control group ( $8.3 \pm 0.10$  Log CFU/Intestine) at post treatment, also that of 250mg/kg/bid herbal remedy treated rats (Group 8:  $4.3 \pm 0.1$  Log CFU/Intestine) and 250mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 10: 0 Log CFU/Intestine) decreased significantly ( $P < 0.001$ ) when compared to the infection control groups ( $8.3 \pm 0.10$  Log CFU/Intestine) at post-treatment. There were no significant differences ( $P > 0.05$ ) in the intestinal bacterial load of the rats when 500 mg/kg/bid and 250 mg/kg/bid herbal remedy were tested singly at post-treatment ( $4.25 \pm 0.05$  Log CFU/Intestine and  $4.3 \pm 0.1$  Log CFU/Intestine, respectively) and in combination with Ciprofloxacin (0 Log CFU/Intestine in each case) (Figure 3).

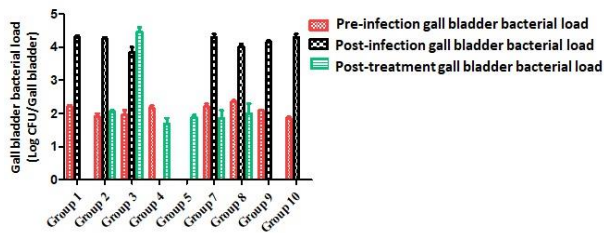


**Figure 3.** A histogram showing the intestinal bacterial load (Log CFU/Intestine) of control and test rats at different intervention phases.

#### 3.3. Effect on gall bladder bacterial load

The gall bladder bacterial load of 500 mg/kg/bid herbal remedy treated rats (Group 7:  $1.85 \pm 0.25$  Log CFU/gall bladder) and 500 mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 9: 0 Log CFU/gall bladder) decreased significantly ( $P < 0.001$ ) when compared to the infection control group ( $4.3 \pm 0.06$  Log CFU/gall bladder) at post-treatment and also that of 250 mg/kg/bid herbal remedy treated rats (Group 8:  $2 \pm 0.30$  Log CFU/gall bladder) and 250 mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 10: 0 Log CFU/gall bladder) decreased significantly ( $P < 0.001$ ) when compared to infectious group ( $4.3 \pm 0.06$  Log CFU/gall bladder). There were no

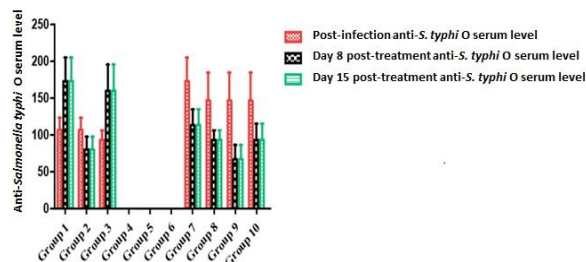
significant differences ( $P>0.05$ ) in the intestinal bacterial load of the rats when 500mg/kg/bid and 250 mg/kg/bid herbal remedy were tested singly at post-treatment ( $1.85\pm 0.25$  Log CFU/gall bladder and  $2\pm 0.30$  Log CFU/gall bladder, respectively) and in combination with Ciprofloxacin (0 Log CFU/gall bladder in each case) (Figure 4).



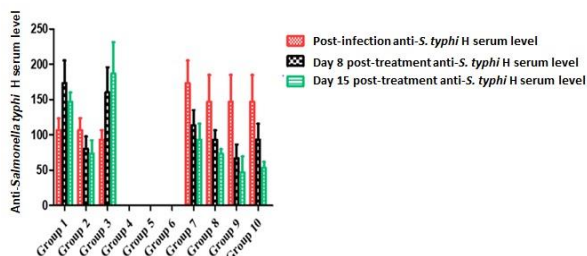
**Figure 4.** A histogram showing the gall bladder bacterial load of control and test rats at different intervention phases.

### 3.4. Effect on anti-*S.typhi* O and H serum levels

The anti-*S.typhi* O and H serum levels at day 8 post-treatment of 500 mg/kg/bid herbal remedy treated rats (Group 7:  $113.3\pm 21.7$ ) decreased non-significantly ( $P>0.05$ ), while 500 mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 9:  $66.6\pm 19.7$ ) decreased significantly ( $P<0.001$ ) when compared with the infection control group ( $173.3\pm 32.1$ ) and also at day 15, 500 mg/kg/bid herbal remedy treated rats post-treatment (Group 7:  $93.3\pm 22.3$ ) decreased significantly ( $P<0.05$ ) and 500 mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 9:  $66.7\pm 19.7$ ) was still significant ( $P<0.05$ ) when compared with infection control group ( $173.3\pm 32.1$ ). There was non-significant decrease ( $P>0.05$ ) in the anti-*S. typhi* O and H serum levels of the rats when 500 mg/kg/bid and 250mg/kg/bid herbal remedy were tested singly at post-treatment (day 8,  $113.3\pm 21.7$  and  $93.3\pm 13.3$ ) and also at post-treatment (day 16,  $93.3\pm 22.3$  and  $73.3\pm 6.7$ ) and in combination with Ciprofloxacin (day 8  $66.6\pm 19.7$  and  $93.3\pm 22.3$ , respectively) and (day 16 ( $66.7\pm 19.7$  and  $93.3\pm 22.3$ , respectively) (Figures 5 and 6).



**Figure 5.** A histogram showing the anti-*S. typhi* O serum level of control and test rats at different intervention phases.

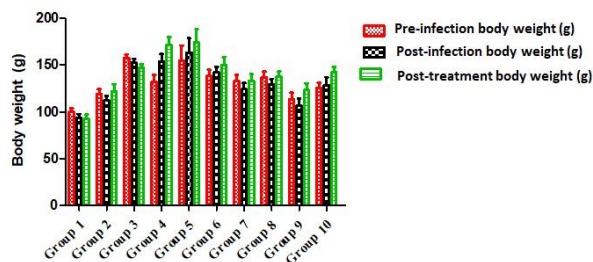


**Figure 6.** A histogram showing the anti-*S. typhi* H serum level of control and test rats at different intervention phases.

### 3.5. Effect on body weight

The body weight of 500mg/kg/bid herbal remedy treated rats (Group 7:  $133.1\pm 7.1$ g) and 500 mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 9:  $123.3\pm 7.2$ g) increased, but it was insignificant ( $P>0.05$ ) when compared to the infection control group ( $92.5\pm 4.5$ g) at post-treatment, however, that of 250 mg/kg/bid herbal remedy treated rats (Group 8:  $137.8\pm 5.4$ g) and 250mg/kg/bid herbal remedy and Ciprofloxacin treated rats (Group 10:  $142.7\pm 6.0$ g) was found to be significant ( $P<0.05$  and  $P<0.01$ , respectively). There were no significant differences ( $P>0.05$ ) in the weight of the rats when 500mg/kg/bid and 250mg/kg/bid herbal remedy were tested singly ( $133.1\pm 7.1$ g and  $137.8\pm 5.4$ g, respectively) and in combination with Ciprofloxacin ( $123.3\pm 7.2$ g and  $142.7\pm 6.0$ g, respectively) (Figure 7).





**Figure 7.** A histogram showing the body weight of control and test rats at different intervention phases.

Symptomatically, the establishment of disease with *S. typhi* was characterized by fever, refusal to feed and inactivity post-infection. Significant increase in the rectal, intestine and gall bladder bacterial load; together with elevated anti-*S. typhi* O and H serum antibodies were used as microbiologic indicators to confirm a successful disease induction in the experimental animals.

Post-treatment, the herbal remedy significantly improved and even cleared symptoms of *S. typhi* infection in the rats. Also, the rectal, intestine and gall bladder bacterial load; together with the anti-*S. typhi* O and H serum antibodies reduced significantly. The ability of the herbal remedy to improve and clear the clinical symptoms of *S. typhi* infection in the test rats (*i.e.*, clinical cure); as well as reduce the bacterial load (*i.e.*, microbiologic cure) underscores the curative potential of the herbal remedy. This is in agreement with the work of Etuk and Francis (2003) who reported on the efficacy of an herbal remedy, aqueous extract of *Psidium guajava* leave in rats infected with *S. typhi*. Adeleye *et al.* (2009) had previously described the antimicrobial effect of Epa Ijebu on several Gram-negative bacteria and this may account for the findings in this study.

Ingestion of faeces contaminated water and food, dirty hands, flies, and meat from infected animals are the most common ways to contract *Salmonella typhi*. Following adequate therapy, clinical cure from *S. typhi* infection is defined as the absence of signs and symptoms associated with *S. typhi* infection after receiving relevant treatment. Immunological cure, on the other hand, is determined by the non-detection of blood and stool antigens, whilst microbiological cure is determined by a negative blood and stool cultures (Enitan *et al.*, 2019). Anti-*S. typhi* immunoglobulins M (IgM) and G (IgG) in the serum, as well as stool antigen, are key immunological markers in the

diagnosis of *S. typhi* infection. Immunologically, anti-*S. typhi* immunoglobulins M (IgM) and G (IgG) can be seen in serum of patients 1-7 days and 7-21 days after exposure to *S. typhi* infection, respectively. While IgM levels fall rapidly, IgG levels tend to last considerably longer, but do not offer lifelong immunity (Enitan *et al.*, 2019).

Furthermore, the detection of serum anti-*S. typhi* IgG and stool *S. typhi* antigen in the absence of serum *S. typhi* IgM is common among convalescent and chronic carriers of the *S. typhi* organism, who, even after no outward signs or symptoms of the disease, have pathogens in their feces, and thus serve as a critical reservoir of infection within the community (Ajayi *et al.*, 2015). In considering the aforementioned, convalescent and chronic typhoid pathogen carriers must be identified and treated in order to break the infection cycle (Enitan *et al.*, 2019).

Unfortunately, this study did not demonstrate the presence of anti-*S. typhi* IgM specific serum antibody which indicates early, primary or current infection with *S. typhi* infection, as well as the anti-*S. typhi* IgG specific serum antibody which indicates late stage, latent or past infection with *S. typhi* infection, due to certain limitations. These limitations affected the confirmation of immunological cure in the experimental animals. However, the study demonstrates a significant reduction in the levels of serum anti-*S. typhi* O and H antibodies with the Widal test, post-treatment with the herbal remedy.

Generally, in clinical practice, the Widal test is a serological assay which tests for the presence of salmonella antibodies in a patient's serum. When culture or antigen testing are not available, the widal test can be useful in diagnosing typhoid and paratyphoid in endemic areas if performed correctly and interpreted with clinical findings (Enitan *et al.*, 2019).

The Widal test determines the amount of agglutinating antibodies to O (somatic) and H (flagellar) antigens. The O agglutinins can usually be discovered 6–8 days after the onset of fever in acute typhoid fever, and the H agglutinins after 10–12 days. A four-fold or larger elevation in acute and convalescent antibody titers is used to diagnose typhoid illness (*i.e.*, between the first and third weeks). Or a single titer that is much greater than the population's mean baseline titer. A diagnostic four-fold rise is uncommon, probably because titres are already highly

elevated when a patient's blood is tested for the first time (Enitan *et al.*, 2019).

The ability to evaluate Widal test results requires knowledge of local normal O and H agglutinin titres. Antibody levels in a healthy population, on the other hand, can change over time and in different places, making it challenging to establish a baseline antibody cut off level in a defined area and community. The Widal test's utility in low-typhoid endemic areas is limited by weak and delayed O and H antibody responses. Variations in the performance and interpretation of Widal tests vary between laboratories, further compromising the test's accuracy. The diagnostic value of the Widal test remains controversial. When only a single acute-phase serum sample is evaluated, which is a normal practice; most believe that the test is not sensitive or specific enough to be clinically relevant. A positive result in a single test does not rule out the possibility of enteric fever, nor does a negative result rule it out (Enitan *et al.*, 2019).

Furthermore, the effect of the herbal remedy ready when used singly was comparable to that of Ciprofloxacin (a conventional antibiotic for treating infection with *S. typhi*). In view of continuous emergence of antibiotic resistance, the herbal remedy may serve as an alternative for the treatment of *S. typhi* infection due to that fact that it is both cheap and readily available especially in resource-limited settings with little or no access to healthcare services.

In addition, the herbal remedy when combined with Ciprofloxacin produced a synergistic effect which is evident by total clearance of bacteria from the intestine and gall bladder of the treated rats (*i.e.*, a sterile culture was obtained after overnight incubation of the organ homogenates on *Salmonella-Shigella* culture media). The result of this study is consistent with the work of Enitan *et al.* (2016) who demonstrated the synergistic potential of methanolic leaf extract of *Plukenetia conophora* Mull. *arg.* when combined with Ciprofloxacin in rats challenged with *Pseudomonas aeruginosa* urinary tract infection. The combination of herbal remedy with antibiotics leading to partial or total synergism has been reported by Didry *et al.* (1992). This explains why various plants are now being used to prevent or treat bacterial illness in parts or whole, or in mixture with other plants or traditional medicines.

#### 4. Conclusion

*Gbogbonise Epa Ijebu* proved to be effective against experimental *S. typhi* infection in rats and when used in combination with standard drug (Ciprofloxacin) gave a synergistic effect. Both clinical and microbiological cure was achieved using *Gbogbonise Epa Ijebu* Herbal remedy in experimental animals infected with *S. typhi*. This gave further credence to the curative potential of the herbal remedy as claimed by both the manufacturer and vendors.

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