Journal of Complementary and Alternative Medical Research

Volume 20, Issue 1, Page 32-54, 2022; Article no.JOCAMR.70491
ISSN: 2456-6276

In-vivo Antibacterial and Synergetic Effect of Garlic (*Allium sativum* Linn) and Water Lilies (*Nymphae lotus* Linn) Extracts on Multi-Drug Resistant Enteric Bacteria

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JOCAMR/2022/v20i1407

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/70491

Original Research Article

Received: 05/06/2022
Accepted: 07/08/2022
Published: 14/12/2022

ABSTRACT

Enteric bacterial pathogens are the major causes of food-bourne diseases and remains a crucial global health concern. The emergence of multi-drug resistant enteric bacterial has been a major public health concern especially in developing countries of the world. The *in-vivo* antibacterial and synergetic effect of garlic (*Allium sativum* Linn) and water lilies (*Nymphae lotus* Linn) extracts on multi-drug resistant enteric bacteria was examined using experimental mice obtained from animal house, College of Medicine, Obafemi Awolowo University, Ile-Ife, Nigeria in accordance with laboratory practice regulation and the principle of humane laboratory animal care. The histological effects of treatment with water extract of *A. sativum*, ethanol extract of *N. lotus* and combination of water extract of *A. sativum* and ethanol extract of *N. lotus* at ratio 1:1 on organs invasion of

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Salmonella showed that major pathological effects were exerted on the liver and kidney of mice. The findings for this study suggests that the extracts of *N. lotus* and *A. sativum* may be used as antibacterial agents in the management of infections that may occur as a result of consumption or contact with faecally impacted surface waters.

Keywords: Enteric bacteria; food-bourne; global health; antimicrobial resistance; In-vivo; antibacterial; garlic (Allium sativum) and water lilies (Nymphae lotus); histological effects.

1. INTRODUCTION

Enteric bacterial pathogens are the major causes of food-borne gastroenteritis in humans and remain important public health problems worldwide. The emergence of Multi-drug resistance (MDR) is a global concern, particularly in developing countries of the world [1]. Multi-drug resistance (MDR) has emerged as one of the principal public health challenges of the 21st century that threatens the effective prevention and treatment of increasing range of infections caused by bacteria, parasites, viruses and fungi [2]. Multidrug-resistant organisms are usually bacteria that have become resistant to the antibiotics used to treat them. The problem of MDR is especially urgent regarding antibiotic resistance in bacteria. Over several decades, to varying degrees, bacteria causing common or severe infections have developed resistance to each new antibiotic. Faced with this reality, the need for action to avert a developing global crisis in health care is imperative. The World Health Organization (WHO) has long recognised the need for an improved and coordinated global effort to contain MDR. In 2001, the WHO Global Strategy for Containment of Antimicrobial Resistance provided a framework of interventions to slow the emergence and reduce the spread of MDR microorganisms [2].

Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. The world is now moving towards the herbal medicine or phytomedicines that repair and strengthening bodily systems (especially the immune system, which can then properly fight foreign invaders) and help to destroy offending pathogens without toxic side effects. The earliest documentation about the usage of herbal remedies comes from China and dates back to 2800 BC [3].

The importance of *N. lotus* is not only historically and ecologically, but also medicinally. It has been reported to cure liver diseases, as antiepileptic, against haematuria and Jaundice and as a sedative and cooling herb [4].

The early Egyptians used garlic to treat diarrhea and its medical power was described on the walls of ancient temples and on papyrus dating to 1500 BC [5].

In Africa, particularly in Nigeria, it is used to treat abdominal discomfort, diarrhea, otitis media and respiratory tract infections [6].

The aim of this study was to determine the *In-vivo* antibacterial and synergetic effect of garlic (*Allium sativum* Linn) and water lilies (*Nymphae lotus* Linn) extracts on multi-drug resistant enteric bacteria.

2. MATERIALS AND METHODS

2.1 Source of Microorganism

Enteric bacteria used in this study were stock cultures from our previous work. They were isolated from Ogbese river water and stored at microbiology department microorganism bank of Federal university of Technology, Akure.

2.2 Source of Plants Extracts

The garlic (*Allium sativum*) and Water lilies (*Nymphae lotus*) extracts used in this study were from 100% stock concentration of *Allium sativum* and *Nymphae lotus* extracts stored at 4°C in a well corked universal bottle. It was reconstituted with DMSO to a required concentration at each use.

2.3 Antibacterial Effect of the Combination of Ethanol Extract of *N. lotus* Root and Water Extract of *A. sativum* at Ratio 1:1 to Enteric Bacterial Isolates

The antimicrobial effect of the combination of ethanol extract of *N. lotus* root and water extract of *A. sativum* at ratio 1:1 on resitant enteric

bacteria isolates were determined using the method described by Omoya and Ajayi [7].

2.3.1 Calculation of fractional inhibitory concentration index (FIC)

The FIC of combined plants extracts was determined by a standard checkerboard method. The FIC was calculated according to the equation [8]: FIC_{index} = MIC of plant extracts in combination/MIC of plant extract alone. FIC of the two plants extracts = FIC index of plant extract A + FIC index of plant extract B. Synergism is defined as FIC ≤ 0.5, additive as FIC > 0.5 and ≤ 1, indifference as FIC > 1 and ≤ 2 and antagonism is defined as FIC > 2 [9].

2.4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Combination of Ethanol Extract of *N. lotus* Root and Water Extract of *A. sativum* at Ratio 1:1 to Enteric Bacteria ISOLATES

The Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined using the broth (tube) dilution technique [10]. Dilutions of the extract in Mueller Hinton broth were prepared in tubes. The concentration of inoculum was also standardized to 0.5 McFarland‟s turbidity, The Mueller Hinton broth in tubes containing the different concentration of the combination of ethanol extract of *N. lotus* root and water extract of *A. sativum* at ratio 1:1, 2.5 mg/ml, 5mg/ml, 10 mg/ml, 20 mg/ml, 40 mg/ml, 80 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml were then inoculated with 0.5 ml of the standardized culture. The tubes were then incubated at 37 °C for 24 hours. MIC and MBC values were recorded [11].

2.5 Preparation of Standard Inoculums for *In-vivo* Assay

The isolates used for *in vitro* assay were prepared for *in vivo* according to the method of Chukwuka et al. [12]. Overnight colonies were transferred to a tube of sterile saline. The bacterial suspensions were compared to the 0.5 McFarland standards against a sheet of white paper on which black lines were drawn. The bacterial suspensions were adjusted to the proper density as the 0.5 McFarland by adding sterile saline for more bacterial growth. Then bacterial suspensions were diluted to obtain 1.5 x 10^8 cfu/ml which is the infectious dose.

2.6 Source of Experimental Swiss Albino Mice

Experimental mice were obtained from animal house, College of Medicine, Obafemi Awolowo University, Ile-Ife, Nigeria and kept in a wooding cage for 14 days for acclimatization under standard environmental conditions with a 12-hour light/dark cycle and were maintained on standard feed (vital feed) and water *ad libitum*. Experimental mice were used in accordance with laboratory practice regulation and the principle of humane laboratory animal care as documented by Zimmermann [13]. They were both sexes weighing between 7-13g. During the acclimatization period, all mice were well fed with water and commercial feed.

2.7 Toxicity Test of Ethanol Extract of *N. lotus*, Water Extract of *A. Sativum* and Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio 1:1 on Experimental Mice

The toxicity was carried out according to method used by Onwusonye et al. [14]. The study was conducted in two phases. A total of 54 mice were used for toxicity of three treatments. About 18 animals per treatment were divided into six groups of three mice each. Each group of animals was administered different doses of extract orogastrically. The mice were placed under observation for 24 hours, 48 hours, and 72hours to monitor their behavior as well as if mortality occurs. Three groups were administered with lower (10, 25 and 50 mg/kg body weight) dose of oil while other three were administered with higher (100, 500 and 1000 mg/kg body weight) dose.

Then the LD_{50} is calculated by the formula:

$$LD_{50} = \sqrt{(D0 \times D100} \quad \text{i.}$$

D0= Highest dose that gave no sign of toxicity
D100= Lowest dose that produced sign of toxicity

2.8 Infection of Experimental Mice with Enteric Bacterial Isolates and Treatment with Extracts of *N. lotus* and *A. sativum*

In-vivo assay was carried out using 6 weeks old Swiss albino mice (n=114) comprising of both the

treatment and control groups., they were divided into 8 groups of 3 mice each and all groups contains both sexes weighing between 9 to 18 g body weight. The Swiss albino mice were infected with the isolated enteric bacteria orogastrically. The enteric bacteria were induced using the method proposed by Pan et al. [15], with modification. The mice were fasted overnight and given, by gavage, 1 mL of saline solution (0.9% NaCl) containing 1.5×10^8 CFU of enteric bacteria, except animals of group 8 (which were neither infected nor treated, hence group 8 was used as neutral control; group 8 received distilled water). Animals of groups 4, 5 and 6 (which were infected, but not treated) received distilled water during the treatment period, hence were used as negative control groups; and those of group 7A, 7B and 7C received ciprofloxacin, and thus were used as positive control groups. Group 1D, 2D 3D were not infected but fed with extract to monitor the effect of extract on the mice, while group 1, 2 and 3 were infected and treated with LD_{50}. They were monitored for physical manifestation of fever associated with ingestion of enteric bacteria for a period of four days. To verify that infection has occurred, the bacterial load of the faeces of the animals were determined one day before infection and during four days following the infection: a steady increase in the bacterial load during the four days indicated the establishment of the infection. The animals were maintained at room temperature [(23 ± 2) ºC] with a 12 h light-dark cycle and standard animal feed and water were provided *ad libitum.* After administration of the bacterial suspension (inoculum), faecal samples were then collected every day and the numbers of the bacteria per gram of faeces were determined (cfu/g). In fact, faeces collected were dissolved in saline distilled water (0.9% NaCl) at the proportion of 1 g for 2 mL of suspension. Aliquots (100 µL) of faecal suspensions were serially diluted in saline distilled water (0.9% NaCl) and then plated on duplicate *Salmonella-Shigella* agar Eosin Methylene Blue agar plates respectively, which were subsequently incubated overnight at 37ºC. Typical colonies were then identified and counted on the plates. Thereafter, the extracts of *N. lotus* and *A. sativum* were used for the treatment of the infected mice.

2.9 Collection of Blood and Preparation of Serum Sample from Experimental Mice

This was done according to the method used by Gatsing et al. [16]. At the end of the treatment period, mice were anaesthetized using chloroform vapour prior to dissection. Blood samples were collected by cardiac puncture into two different tubes, one containing anticoagulant ethylenediaminetetraacetic acid (EDTA) and the other without anticoagulant. The blood was used for the determination of haematological parameters.

2.10 Measurement of the Percentage Weight Gain of Experimental Mice

Weights of the mice were taken daily using a digital weighing balance. The mice were demobilized and fixed in a container while taking note of the weight of the container. The weight increase or weight loss of animals was evaluated and the percentage of weight gain was determined.

2.11 Temperature Evaluation of Experimental Mice

Temperatures of the mice were checked daily using a clinical thermometer fixed into their anus for 30 seconds after demobilizing them in a fixed container. The readings were taken daily for the period of the experiment.

2.12 Histopathological Analysis of Experimental Mice

Tissue cross sections were prepared and analyzed using conventional techniques described by Khalid et al. [17]. After sacrificing the animals, small pieces of liver were fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissue was embedded in paraffin wax and sectioned into five micrometres thick with the rotary microtome, then stained with hematoxylin and eosin. Then the sections were examined with light microscope and photographed using a microscopic camera.

2.13 Statistical Analysis

Data obtained were expressed as mean ± Standard Error of Mean and were statistically analysed using One Way Analysis of Variance (ANOVA). The new Duncan Multiple Range test was used to separate and compare means of different groups. A *P*-value of < 0.05 was considered statistically significant.

3. RESULTS

3.1 Sensitivity Pattern of Bacterial Isolates to Combination of Ethanol Extract of *N. lotus* Root and Water extract of *A. sativum* at Ratio 1:1

The combined extracts inhibit all the resistant enteric bacteria at 12.50 mg/ml. The highest mean zone of inhibition was recorded as 25.00 ± 0.58 mm at 100 mg/ml concentration which was significantly (P< 0.05) higher than mean zone of inhibitions of individual extracts (Table 1).

3.2 Antimicrobial Effect of the Combination of Ethanol Extract of *N lotus* Root and Water Extract of *A sativum* at ratio 1:1 on Enteric Bacterial Isolates

The interaction of ethanol extract of *N lotus* root in combination with water extract of *A sativum* as antimicrobial against resistant enteric bacteria isolates at ratio 1:1 was analysed and calculated. FIC_{index} = MIC of plants extracts in combination /MIC of plants extracts alone; FIC of the two extracts = FIC_{index} of extract A + FIC_{index} of extract B. Synergism is taken as FIC ≤ 0.5, additive as FIC > 0.5 and ≤ 1, indifference as FIC > 1 and ≤ 2 and antagonism is defined as FIC > 2. The combination of the two extracts at ratio 1:1 is indifference for all resistant enteric bacteria isolates Table 2.

3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Combination of Ethanol Extract of *N. lotus* Root and Water Extract of *A. sativum* at Ratio 1:1 to Enteric Bacteria ISOLATES

The Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentration (MBC) of the combination of ethanol extract of *N. lotus* root and water extract of *A. sativum* at ratio 1:1 to resistant enteric bacteria Isolates is 10mg/ml and 20mg/ml respectively for all test isolates (Table 3).

Table 1. Sensitivity pattern of bacterial isolates to Combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio 1:1

Extracts	Concentration	Mean zone of inhibition (mm) *E. coli*	Mean zone of inhibition (mm) Salmonella	Mean zone of inhibition (mm) Shigella
Combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio 1:1	100 mg/ml	15.00±0.58^{c}	25.00±0.58^{d}	18.67±0.67^{c}
	50 mg/ml	14.67±0.33^{c}	22.00±1.16^{c}	16.67±0.67^{c}
	25 mg/ml	12.33±0.33^{b}	14.00±1.16^{ab}	12.67±0.67^{b}
	12.50 mg/ml	8.67±0.67^{a}	12.67±0.67^{a}	7.67±1.20^{a}
	Positive Control	17.67±0.33^{d}	16.67±0.88^{b}	16.33±0.88^{c}

Key: Positive control- ciprofloxacin (0.63 mg/ml)

Legend: Values are means ± SEM (Standard error of mean) of triplicates, values in the same row carry same superscript are not significantly different according to new Duncan's multiple range test at p ≤ 0.05

Table 2. Interpretation of antimicrobial effect of the combination of ethanol extract of *N. lotus* root and water extract of *A. sativum* at ratio 1:1 to enteric bacteria isolates

Bacterial isolate	FIC-index root ethanol	FIC-index garlic water	FIC	Interpretation
Escherichia coli	1	0.5	1.5	Indifference
Salmonella	1	1	2	Indifference
Shigella	1	1	2	Indifference

Legend: *The FIC of combined plants extracts was determined by a standard checkerboard method. The FIC was calculated according to the equation; FIC_{index} = MIC of plants extracts in combination /MIC of plants extracts alone; FIC of the two extracts = FIC_{index} of extract A + FIC_{index} of extract B.*

Synergism is defined as FIC ≤ 0.5, additive as FIC > 0.5 and ≤ 1, indifference as FIC > 1 and ≤ 2 and antagonism is defined as FIC > 2

KEY: *FIC-Fractional inhibitory concentration*

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the combination of ethanol extract of *Nymphaea lotus* root and water extract of *A. sativum* at ratio 1:1 to enteric bacteria isolates

S/N	Bacteria Isolates	MIC (mg/ml)	MBC (mg/ml)
1	*Escherichia coli*	10	20
2	*Salmonella*	10	20
3	*Shigella*	10	20

Table 4. Toxicity effect of water extract of *A. sativum* on Swiss Albino Mice

Dosage/body weight (kg)	Day 1	Day 3	Day 5	Day 7
10 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
25 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
50 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
100 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
500 mg/ml/kg	Very active and no sign of toxicity	Hyperactive and no sign of toxicity	Hyperactive, Anorexia was observed	Not Active
1000 mg/ml/kg	Very active, and no sign of toxicity	Hyperactive,, Anorexia was observed	Hyperactive, Anorexia was observed	Hyperactive, Anorexia was observed

Acute toxicity was calculated to be 223.61 mg/ml/kg

Table 5. Toxicity effect of ethanol extract of *N. lotus* root on Albino Mice

Dosage/body weight (kg)	Day 1	Day 3	Day 5	Day 7
10 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
25 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
50 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
100 mg/ml/kg	Very active and no sign of toxicity	Not active	Very active and no sign of toxicity	Very active and no sign of toxicity
500 mg/ml/kg	Very active and no sign of toxicity	Not active and Anorexia was observed	Not active and Anorexia was observed	Not Active, Anorexia was observed
1000 mg/ml/kg	Very active and no sign of toxicity	Not active and Anorexia was observed	Not active and Anorexia was observed	Not Active, Anorexia was observed

Acute toxicity was calculated to be 70.71 mg/ml/kg

3.4 Toxicity Effect of Water Extract of *A. sativum* on Swiss Albino Mice

The acute toxicity effect of water extract of *A. sativum* was carried out for seven days and at the end of seven days, it was observed at concentration of 10 and 100 mg/ml/kg body weight of experimental mice that the mice were very active and no sign of toxicity; however, at concentrations of 500 and 1000 mg/ml/kg body weight of experimental mice, the mice were hyperactive and anorexia was observed. There was no mortality during the seven days acute toxicity assay and acute toxicity concentration was calculated to be 223.61 mg/ml/kg Table 4.

3.5 Toxicity Effect of Ethanol Extract of *N. lotus* Root on Albino Mice

Acute toxicity effect of water extract of ethanol extract of *N. lotus* root on Swiss Albino Mice was assayed *in vivo* and the result is shown in Table 5. The acute toxicity effect of ethanol extract of *N. lotus* root was carried out for seven days and at the end of seven days, it was observed at concentration of 10 and 50 mg/ml/kg body weight of experimental mice that the mice were very active and no sign of toxicity however, at concentrations of 500 and 1000 mg/ml/kg body weight of experimental mice, most of the mice were not active and anorexia was observed. There was mortality at concentration of 500 mg/ ml/ kg body weight of experimental mice during seven days acute toxicity assay and acute toxicity concentration was calculated to be 70.71 mg/ml/kg.

3.6 Toxicity Effect of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root in Combination at Ratio (1:1) on Albino Mice

Acute toxicity effect of water extract of ethanol extract of *N. lotus* root on Swiss Albino Mice was assayed *In vivo* and the result is shown in Table 6. The acute toxicity effect of water extract of *A. sativum* and ethanol extract of *N. lotus* root in combination at ratio (1:1) was carried out for seven days and at the end of seven days, it was observed at concentration of 10 and 50 mg/ml/kg body weight of experimental mice that the mice were very active and no sign of toxicity however, at concentrations of 500 and 1000 mg/ml/kg body weight of experimental mice, most of the mice were very active and anorexia were observed. There was no mortality during the seven days acute toxicity assay and acute toxicity concentration was calculated to be 70.71 mg/ml/kg.

Table 6. Toxicity effect of water extract of *A. sativum* and ethanol extract of *N. lotus* root in combination at ratio (1:1) on Albino Mice

Dosage/ body weight (kg)	Day 1	Day 3	Day 5	Day 7
10 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
25 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
50 mg/ml/kg	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity	Very active and no sign of toxicity
100 mg/ml/kg	Very active and no sign of toxicity	Very active, anorexia was observed	Very active, anorexia was observed	Very active and no sign of toxicity
500 mg/ml/kg	Very active and no sign of toxicity	Not active	Very active, anorexia was observed	Active
1000 mg/ml/kg	Very active and no sign of toxicity	Not active	Very active, anorexia was observed	Not Active

Acute toxicity was calculated to be 70.71 mg/ml/kg

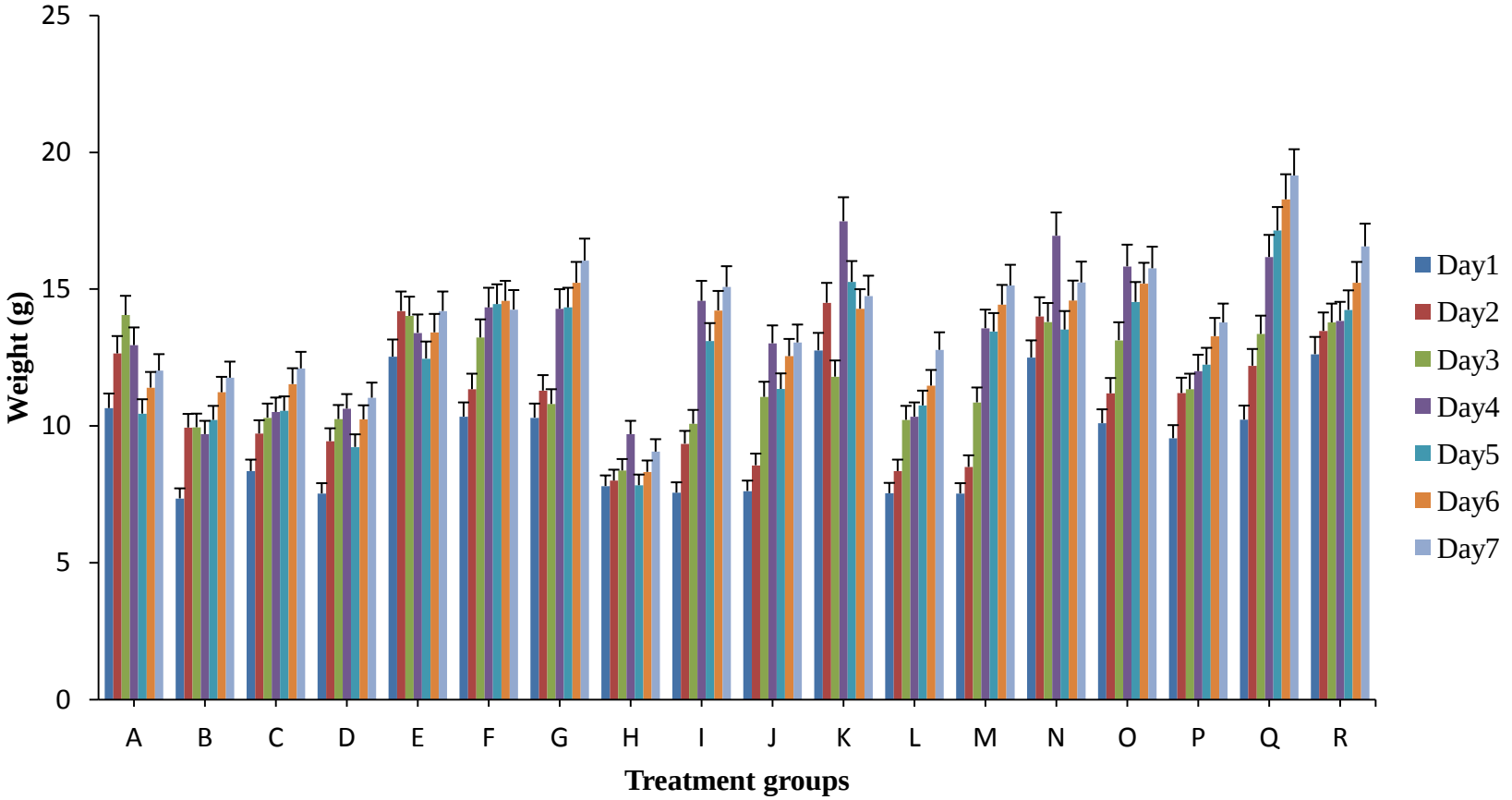

Fig. 1. Change in weight (g) of experimental animals during acute toxicity test

Key : *GW-Water extract of A. sativum, RE- Ethanol extract of N. lotus root, SYN-Combination of water extract of A. sativum and ethanol extract of N. lotus root at ratio 1:1,* ***A****- GW(1000mg),* ***B****- GW(500mg),* ***C****- GW(100mg),* ***D****- GW(50mg),* ***E****- GW(25mg),* ***F****- GW(10mg),* ***G****- RE (1000mg),* ***H****- RE (500mg),* ***I****- RE (100mg),* ***J****- RE (50mg),* ***K****- RE (25mg),* ***L****- RE (10mg),* ***M****- SYN (1000mg),* ***N****- SYN (500mg),* ***O****- SYN (100mg),* ***P****- SYN (50mg),* ***Q****- SYN (25mg),* ***R****- SYN (10mg) /ml/mice*

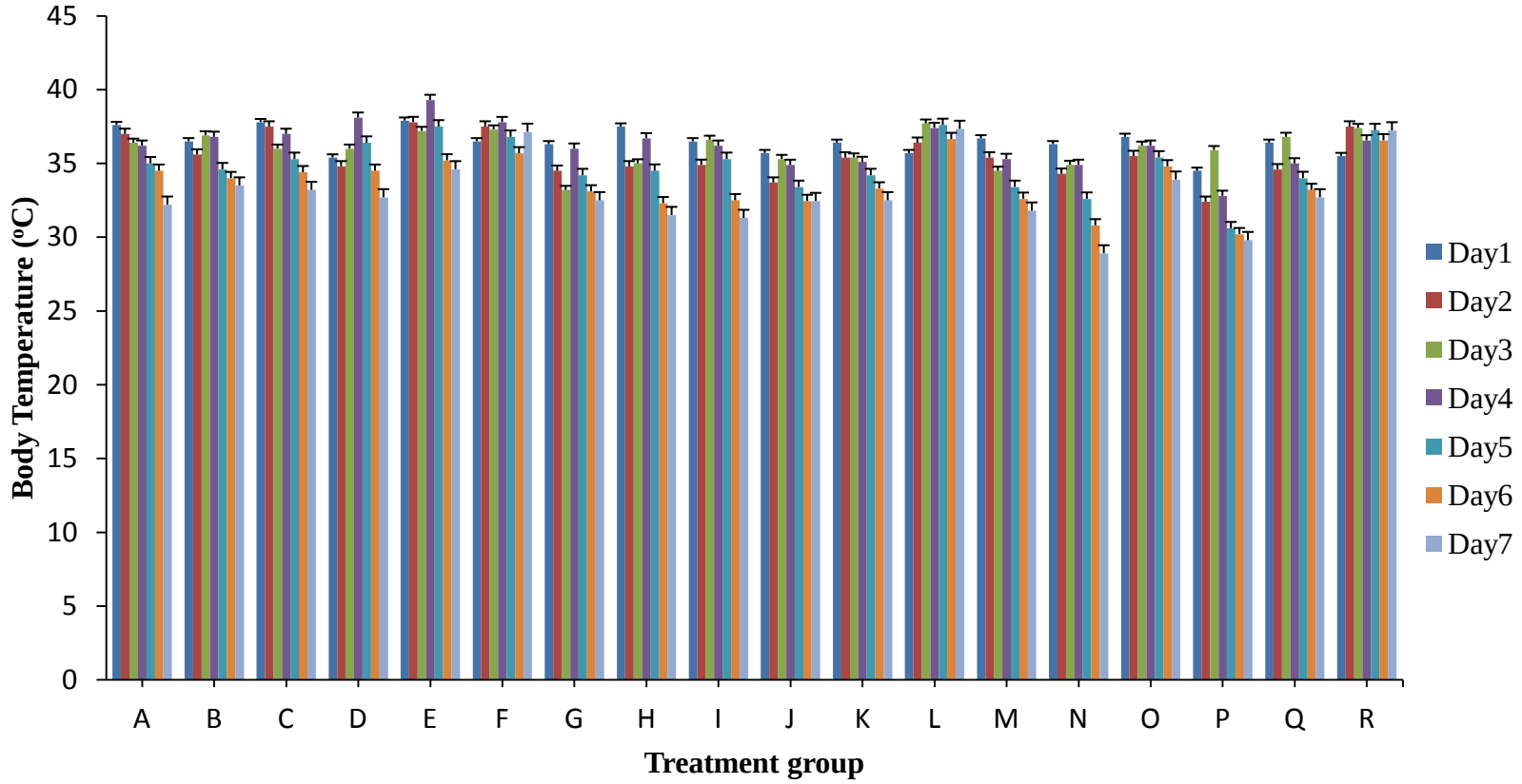

Fig. 2. Change in body temperature (°C) of experimental animals during acute toxicity test

Key: ***GW****-Water extract of A. sativum, RE- Ethanol extract of N. lotus root, SYN-Combination of water extract of A. sativum and ethanol extract of N. lotus root at ratio 1:1,* ***A****- GW(1000mg),* ***B****- GW(500mg),* ***C****- GW(100mg),* ***D****- GW(50mg),* ***E****- GW(25mg),* ***F****- GW(10mg),* ***G****- RE (1000mg),* ***H****- RE (500mg),* ***I****- RE (100mg),* ***J****- RE (50mg),* ***K****- RE (25mg),* ***L****- RE (10mg),* ***M****- SYN (1000mg),* ***N****- SYN (500mg),* ***O****- SYN (100mg),* ***P****- SYN (50mg),* ***Q****- SYN (25mg),* ***R****- SYN (10mg) /ml/mice*

3.7 Change in Weight (g) of Experimental Animals during Acute Toxicity Test

Change in weight (g) of experimental animals during acute toxicity test is shown in Fig. 1. It was observed that all the mice had significant ($p<0.05$) weight gain with the highest weight gain observed at concentration of 25 mg/ml/kg (10.23±0.64 to 19.15±1.15) of body weight of the combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio (1:1).

3.8 Change in Body Temperature (^oC) of Experimental Animals during Acute Toxicity Test

Change in body temperature (^oC) of experimental animals during acute toxicity test is presented in Fig. 2. It was observed that at day seven, extract contributed to significant ($p<0.05$) decrease in body temperature of experimental mice at concentration of 25-1000mg/ml/kg of body weight of all extracts.

3.9 Body Weight Gain Trend for Swiss Albino Mice Treated with Water Extract of *Allium sativum*, Ethanol Extract of *N. lotus* Root and Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio (1:1)

Body weight gain trend for Swiss Albino mice treated with extract of *A. sativum*, ethanol extract of *N. lotus* root and combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio (1:1) respectively **i**s shown in Fig. 3. The result revealed that there was significant ($p<0.05$) change in the body weight of the mice in group 1 to 3 (infected and treated with extracts), 1D to 3D (not infected but fed with extract) and group 8 (were neither infected nor treated). A significant ($p<0.05$) increase in body weight was observed in the group 1D to 3D (not infected but fed with extract) and group 8 (were neither infected nor treated) and significant ($p<0.05$) decrease in body weight was observed in group 1-3 (infected and treated with extracts) and group 4-6 (were infected, but not treated) at the end of the treatment period.

3.10 Change in Body Temperature (^oC) of Experimental Animals during Treatment

Change in body temperature (^oC) of experimental animals during treatment is presented in Fig. 4. It was observed that at day six, extract contributed to significant ($p<0.05$) decrease in body temperature of most of experimental mice in treatment groups (Group 1 to 3) at each extract's acute toxicity concentration of body weight.

3.11 Effects of Treatment with Water Extract of *Allium sativum*, Ethanol Extract of *N. lotus* Root and Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio (1:1) on Faecal Shedding of *Escherichia coli*, *Salmonella* and *Shigella* (CFU/g)

Effects of treatment with water extract of *A. sativum* extract on faecal shedding of *Escherichia coli*, *Salmonella,* and *Shigella* (CFU*/*g) is shown in Fig. 5. The result showed that the faecal shedding of the groups infected and treated with extracts (Group 1A-1C) increased significantly ($p<0.05$) from (5×10^6 cfu/g to 40×10^6 cfu/g), (6×10^6 cfu/g to 56×10^6 cfu/g) and (7×10^6 cfu/g to 29×10^6 cfu/g) respectively before treatment and reduced significantly ($p<0.05$) after three days treatment comparatively, the groups treated with 2 mg/ml/kg of ciprofloxacin (Group 7A-7C) had least reduction after treatment. Effects of treatment with ethanol extract of *N. lotus* root on faecal shedding of *Escherichia coli*, *Salmonella,* and *Shigella* (CFU*/*g) Fig. 6. The result showed that the faecal shedding of the groups infected and treated with extracts (Group2A-2C) increased significantly ($p<0.05$) from (5×10^6 cfu/g to 38×10^6 cfu/g), (8×10^6 cfu/g to 46×10^6 cfu/g) and (9×10^6 cfu/g to 81×10^6 cfu/g) respectively before treatment and reduced significantly ($p<0.05$) after three days treatment comparatively, the groups treated with 2 mg/ml/kg of ciprofloxacin (Group 7A-7C) had least reduction after treatment. Effects of treatment combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio (1:1)on faecal shedding of *Escherichia coli, Salmonella, and Shigella* (CFU/g) Fig. 7. The result showed that the faecal shedding of the groups infected and treated with extracts (Group3A-3C) increased significantly ($p<0.05$) from (7×10^6 cfu/g to 48×10^6 cfu/g), (7×10^6 cfu/g to 126×10^6 cfu/g) and (8×10^6 cfu/g to 43×10^6 cfu/g) respectively before treatment and reduced significantly ($p<0.05$) after three days treatment comparatively, the groups treated with 2 mg/ml/kg of ciprofloxacin (Group 7A-7C) had least reduction after treatment.

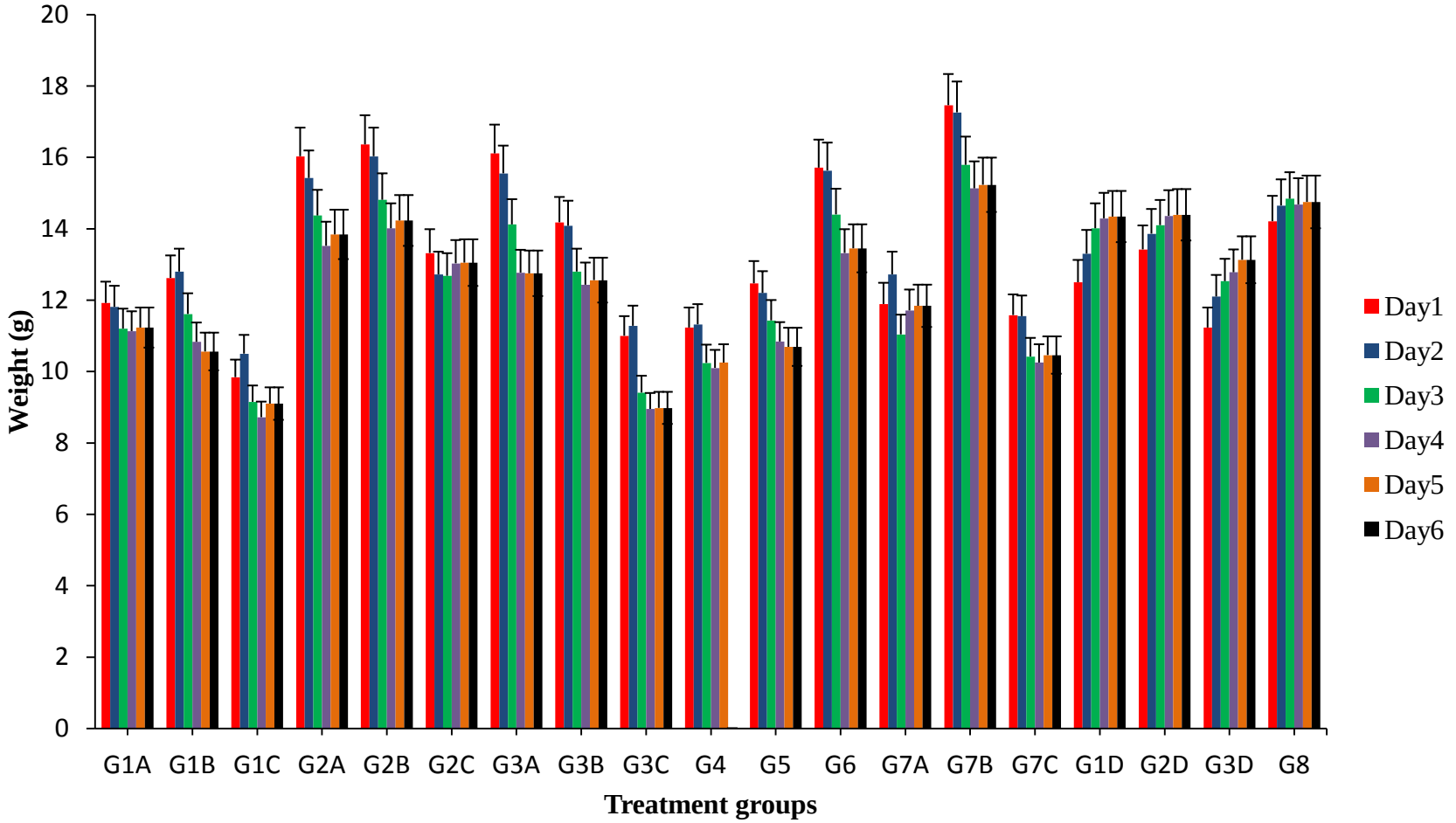

Fig. 3. Body weight gain trend for Swiss albino mice treated with water extract of *Allium sativum*, ethanol extract of *N. lotus* root and combination of water extract of *A. sativum* and ethanol extract of *N. lotus* (Root) at ratio 1:1 respectively

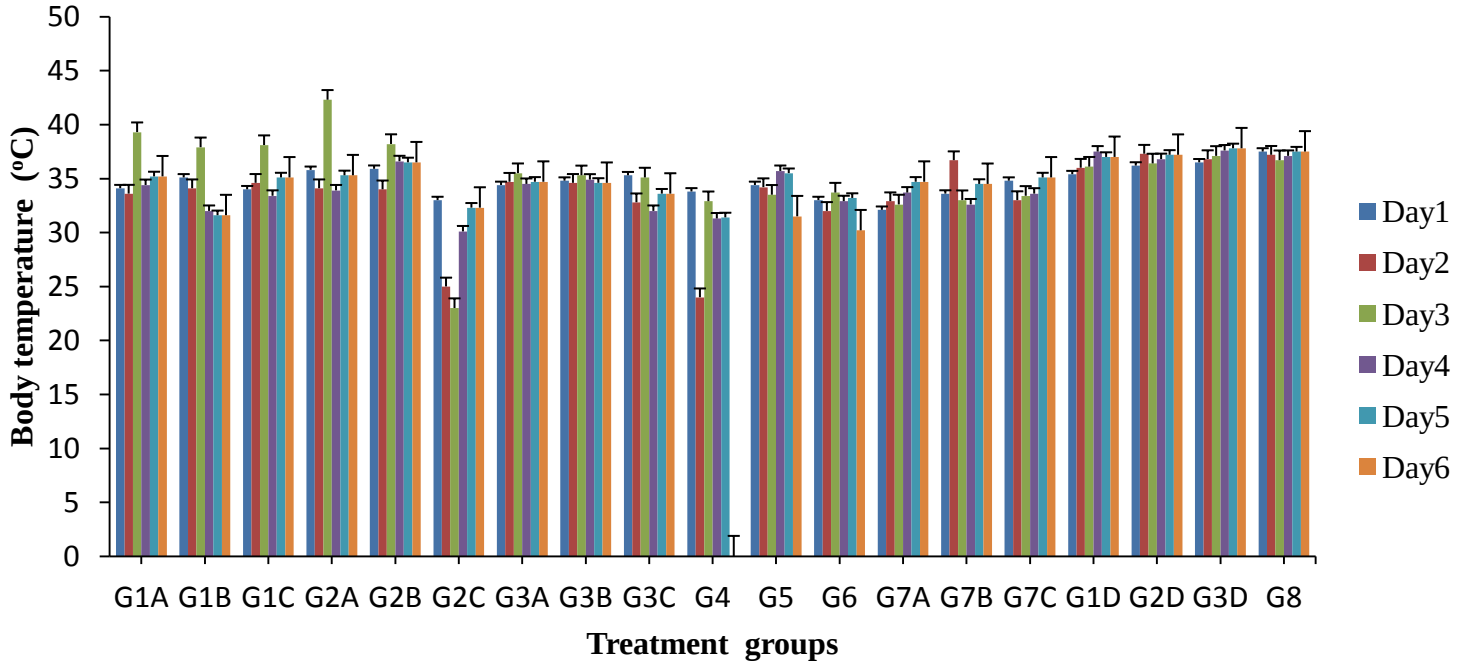

Fig. 4. Change in body temperature (°C) of experimental animals during treatmentKEY: G-GROUP

Group 1A, 1B & 1C *were infected with E. coli, Salmonella and Shigella respectively and treated with water extract of A. sativum (223.61mg/ml/body weight (kg)*
Group 1D *were not infected but fed with water extract of A. sativum (223.61 mg/ml/body weight (kg)*
Group 2A, 2B & 2C *were infected with E. coli, Salmonella and Shigella respectively and treated with ethanol extract of N. lotus root (70.71mg/ml/body weight (kg)*
Group 2D *were not infected but fed with ethanol extract of N. lotus root (70.71 mg/ml/body weight (kg)*
Group 3A, 3B & 3C *were infected with E. coli, Salmonella and Shigella respectively and treated with combination of water extract of A. sativum and ethanol extract of N. lotus(Root) at ratio 1:1 respectively (70.71mg/ml/body weight (kg)*
Group 3D *were not infected but fed with combination of water extract of A. sativum and ethanol extract of N. lotus(Root) at ratio 1:1 respectively (70.71mg/ml/body weight (kg)*
Groups 4, 5 & 6 *were infected with E. coli, Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*
Group 7A, 7B & 7C *were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.*
Group 8 *were neither infected nor treated (they received distilled water during the treatment period)*

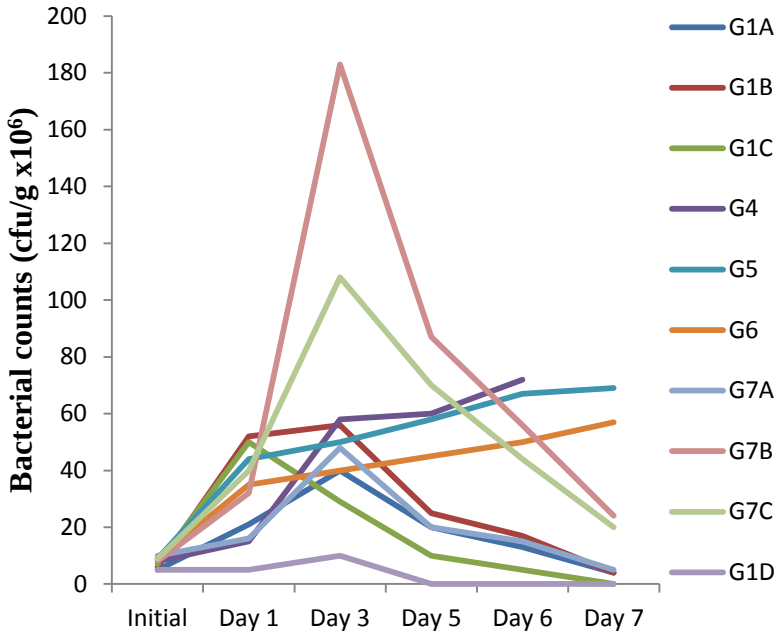

Fig. 5. Effects of treatment with *A. sativum* water extract on faecal shedding of *E. coli*, *Salmonella* and *Shigella* (CFU/g)

Note: initial = colony counts before infection, day 1-3= colony counts during infection, day 5-7 = colony counts during treatment

KEY: G-GROUP

***Group 1A, 1B & 1C** were infected with E. coli, Salmonella and Shigella respectively and treated with extract (223.61mg/ml/body weight (kg)*

***Group 1D** were not infected but fed with extract (223.61mg/ml/body weight (kg) **Groups 4, 5 & 6** were infected with E. coli, Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*

***Group 7A, 7B & 7C** were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.*

3.12 Effects of Water Extract of *Allium sativum*, Ethanol Extract of *N. lotus* Root and Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio (1:1) Extracts on Haematological Parameters of Swiss Albino Mice Infected with *Escherichia coli*, *Salmonella* and *Shigella*

Effects of water extract of *A. sativum* extract on haematological parameters of Swiss Albino mice infected with *Escherichia coli*, *Salmonella* and *Shigella* are revealed in Fig. 8. There was no significant difference between the PCV of mice in group 1A-1C (were infected and treated with extract), 1D (was not infected but fed with extract), and 8 (were neither infected nor treated). Effects of ethanol extract of *N. lotus*root on Haematological parameters of Swiss Albino mice infected with *Escherichia coli*, *Salmonella* and *Shigella* are revealed in Fig. 9. There was no significant difference between the PCV of mice in group 2A-2C (were infected and treated with extract), 2D (was not infected but fed with extract), and 8 (were neither infected nor treated). Effects of combination of water extract of *A. sativum* and ethanol extract of *N. lotus*root at ratio (1:1) extracts on Haematological parameters of Swiss Albino mice infected with *Escherichia coli*, *Salmonella* and *Shigella* are revealed in Fig. 8. There was no significant difference between the PCV of mice in group 3A-3C (were infected and treated with extract), 3D (were not infected but fed with extract), and 8 (were neither infected nor treated).

The highest haematocrit (HCT) was observed in Group 3D 51% (were not infected but fed with extract) which is a little bit above the normal range of haematocrit in mice (36-46 %) and the least in Group 7B 19.2% (infected with *Salmonella* and treated with ciprofloxacin) which

is far below the normal range of haematocrit in mice. The highest white blood cell (WBC) was noted in group 5 (6.6 x 10^3 µl) which was within the normal range of WBC in mice (WBC of 2000 -10000 µl) while the least was observed in group 3B (infected with *Salmonella* and treated with Combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root in at ratio (1:1) (1.6 x 10^3 µl) which was below the normal range of mice WBC.

3.13 Effects of Water Extract of *A. sativum* on Differential white Blood Cell Counts of Albino Mice Infected with *E. coli, Salmonella* and *Shigella* Respectively

Effects of water extract of *A. sativum* on Differential White Blood Cell counts on Swiss albino mice infected with *Escherichia coli*, *Salmonella* and *Shigella* is shown in Fig. 11. The result showed that group 1 D (were not infected but fed with extract (223.61mg/ml/body weight (kg) has the highest lymphocyte cell counts (62.65%) which was within the normal range of mice lymphocyte cell counts (65 – 87%) and group 6 (were infected with *Shigella* but not treated (received sterile distilled water during the treatment period) has the lowest lymphocyte cell counts (50.34%) which was below the normal range of mice lymphocyte cell counts, while group 4 (were infected with *E. coli* but not treated (received sterile distilled water during the treatment period) has the highest percentage neutrophil (37.43%) and (were infected with *E. coli* but not treated (received sterile distilled water during the treatment period) has the highest percentage neutrophil (37.43%).

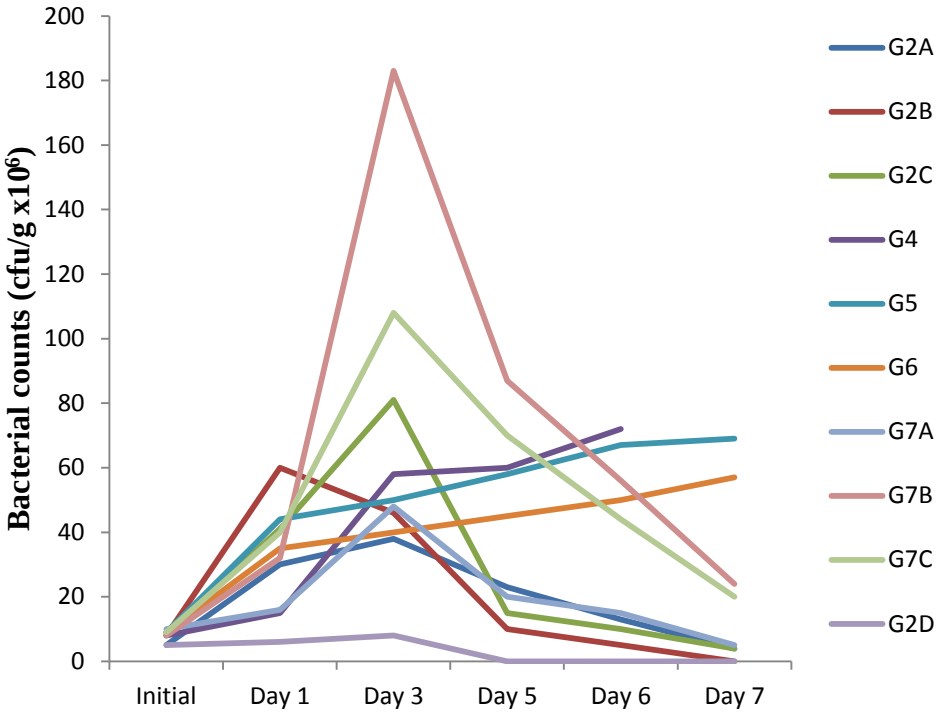

Fig. 6. Effects of treatment with *N. lotus* (Root)ethanol extract on faecal shedding of *E. coli, Salmonella* and *Shigella* (CFU/g)

Note: initial = colony counts before infection, day 1-3= colony counts during infection, day 5-7 = colony counts during treatment

KEY: G-GROUP

***Group 2A, 2B & 2C** were infected with E. coli, Salmonella and Shigella respectively and treated with extract (70.71mg/ml/body weight (kg)*

***Group 2D** were not infected but fed with extract (70.71mg/ml/body weight (kg)*

***Groups 4, 5 & 6** were infected with E. coli, Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*

***Group 7A, 7B & 7C** were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups*

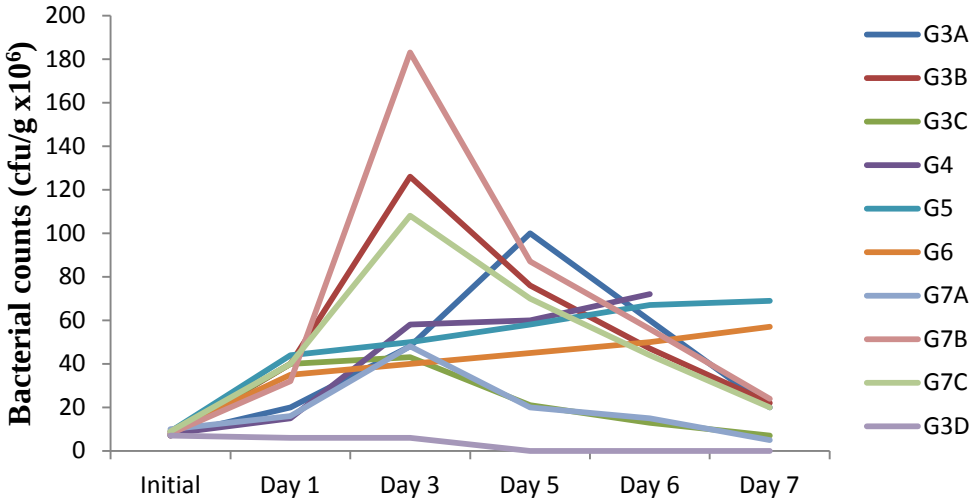

Fig. 7. Effects of treatment with combination of *A. sativum* water extract and *N. lotus* (Root) ethanol extract in ratio 1:1 on faecal shedding of *E. coli*, *Salmonella* and *Shigella* (CFU/g)

Note: initial = colony counts before infection, day 1-3= colony counts during infection, day 5-7 = colony counts during treatment

KEY: G-GROUP

***Group 3A, 3B & 3C** were infected with E. coli, Salmonella and Shigella respectively and treated with extract (70.71mg/ml/body weight (kg)*

***Group 3D** were not infected but fed with extract (70.71mg/ml/body weight (kg)*

***Group 4, 5 & 6** were infected with E. coli,Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*

***Group 7A, 7B & 7C** were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups*

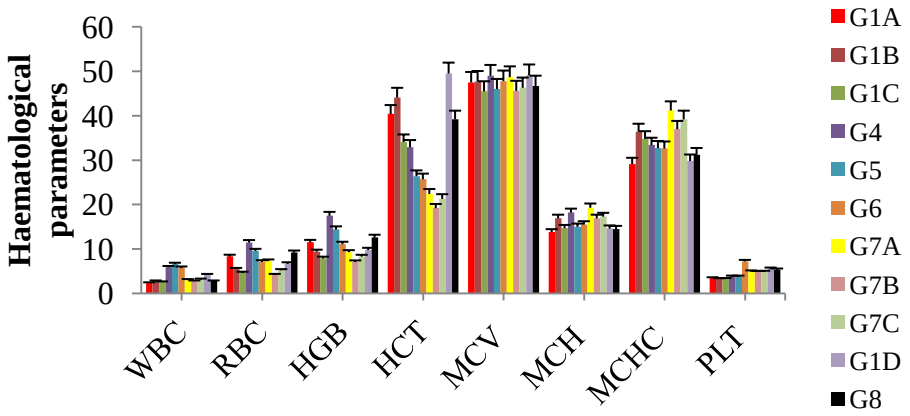

Fig. 8. Effects of water extract of *A. sativum* on haematological parameters of albino mice infected with *E. coli*, *Salmonella* and *Shigella* respectively

KEY: G-GROUP

***Group 1A**, **1B & 1C** were infected with E. coli, Salmonella and Shigella respectively and treated with extract (223.61mg/ml/body weight (kg)*

***Group 1D** were not infected but fed with extract (223.61mg/ml/body weight (kg)*

***Groups 4, 5 & 6** were infected with E. coli, Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*

***Group 7A, 7B & 7C** were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.*

***Group 8** were neither infected nor treated (they received distilled water during the treatment period) **WBC**-White blood cell (/µL×10^3), **RBC**- Red blood cell count(/µ×10^6), **HGB**-Haemoglobin concentration (g/dL), **HCT**-Hematocrit packed cell volume (%), **MCV**- Mean corpuscular volume (fL), **MCH**-Mean corpuscular hemoglobin (Pg), **MCHC**- Mean corpuscular hemoglobin concentration (g/dL), **PLT**-Platelet (/µL×10^5)*

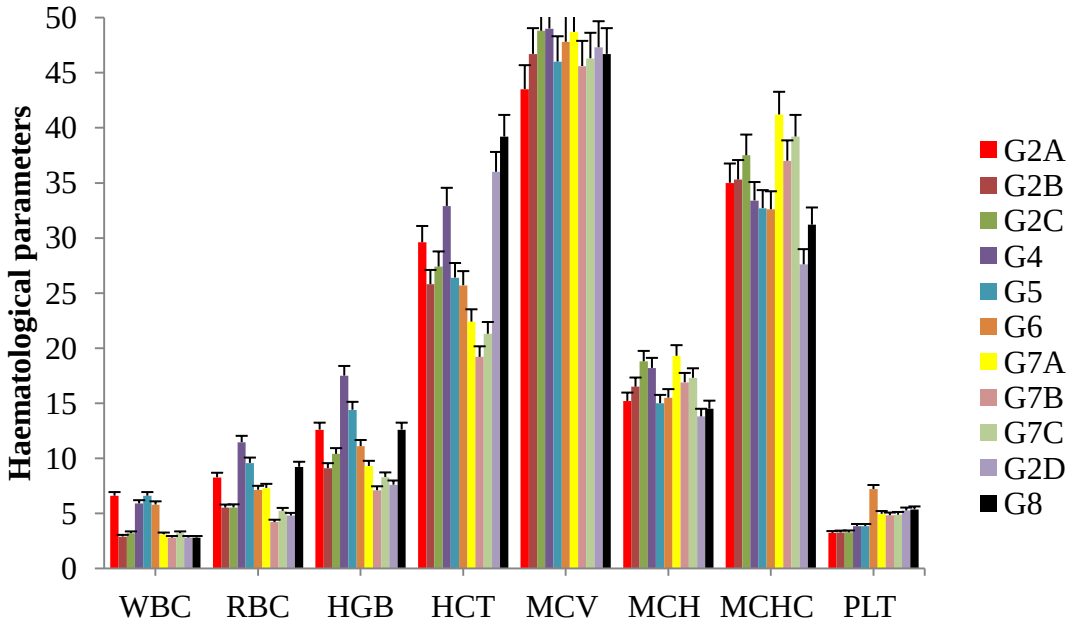

Fig. 9. Effects of ethanol extract of *N. lotus* root on haematological parameters of albino mice infected with *E. coli, Salmonella* and *Shigella* respectively

KEY: G-GROUP

Group 2A, 2B & 2C *were infected with E. coli, Salmonella and Shigella respectively and treated with extract (70.71mg/ml/body weight (kg)*

Group 2D *were not infected but fed with extract (70.71mg/ml/body weight (kg)*

Groups 4, 5 & 6 *were infected with E. coli, Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*

Group 7A, 7B & 7C *were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.*

Group 8 *were neither infected nor treated (they received distilled water during the treatment period)*

WBC*-White blood cell (/µL×10^3), RBC- Red blood cell count(/µ×10^6),* ***HGB-*** *Haemoglobin concentration (g/dL),* ***HCT****-Hematocrit packed cell volume (%),* ***MCV-*** *Mean corpuscular volume (fL),* ***MCH****-Mean corpuscular hemoglobin (Pg),* ***MCHC****- Mean corpuscular hemoglobin concentration (g/dL),* ***PLT****-Platelet (/µL×10^5)*

3.14 Effects of Ethanol Extract of *N. lotus* Root on Differential White Blood Cell Counts of Albino Mice Infected with *E. coli, Salmonella* and *Shigella* respectively

Effects of ethanol extract of *N. lotus* root on Differential White Blood Cell counts on Swiss albino mice infected with *Escherichia coli*, *Salmonella* and *Shigella* is shown in Fig. 12. The result showed that group 2D (were not infected but fed with extract (70.71mg/ml/body weight (kg) has the highest lymphocyte cell counts (65.54%) which was within the normal range of mice lymphocyte cell counts (65 – 87%) and group 6 (were infected with *Shigella* but not treated (received sterile distilled water during the treatment period) has the lowest lymphocyte cell counts (50.34%) which was below the normal range of mice lymphocyte cell counts, while group 4 (were infected with *E. coli* but not treated (received sterile distilled water during the treatment period) has the highest percentage neutrophil (37.43%) and group 8 (were neither infected nor treated (they received distilled water during the treatment period) has the least percentage neutrophil (28.68%) which were both above the normal range of mice neutrophil (7.5 – 27%).

3.15 Effects of Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio 1:1 on Differential White Blood Cell Counts of Albino Mice Infected with *E. coli, Salmonella* and *Shigella* Respectively

Effects of combination of water extract of *A. sativum* and ethanol extract of *N. lotus* (Root) at ratio 1:1 on differential white blood cell counts on Swiss albino mice infected with *Escherichia coli*,

Salmonella and *Shigella* is shown in Fig. 13. The result showed that group 3D (were not infected but fed with extract (70.71 mg/ml/body weight (kg) has the highest lymphocyte cell counts (67.36%) which was within the normal range of mice lymphocyte cell counts (65 – 87%) and group 6 (were infected with *Shigella* but not treated (received sterile distilled water during the treatment period) has the lowest lymphocyte cell counts (50.34%) which was below the normal range of mice lymphocyte cell counts, while group 4 (were infected with *E. coli* but not treated (received sterile distilled water during the treatment period) has the highest percentage neutrophil (37.43%) and group 8 (were neither infected nor treated (they received distilled water during the treatment period) has the least percentage neutrophil (28.68%) which were both above the normal range of mice neutrophil (7.5 – 27%).

3.16 Histological Effects of Treatment with Water Extract of *A. sativum*, Ethanol Extract of *N. lotus* Root and Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio 1:1 on Organs Invasion of *Salmonella* as a Representative for Other Enteric Bacteria

Histological effects of treatment with ethanol extract of *N. lotus* root, water extract of *A. sativum* and combination of water extract of *A. sativum* and ethanol extract of *N. lotus* (Root) at ratio 1:1 on the liver and kidney of Swiss albino mice infected with *Salmonella* revealed major pathological effects were exerted by treatments on the liver and kidney of mice.

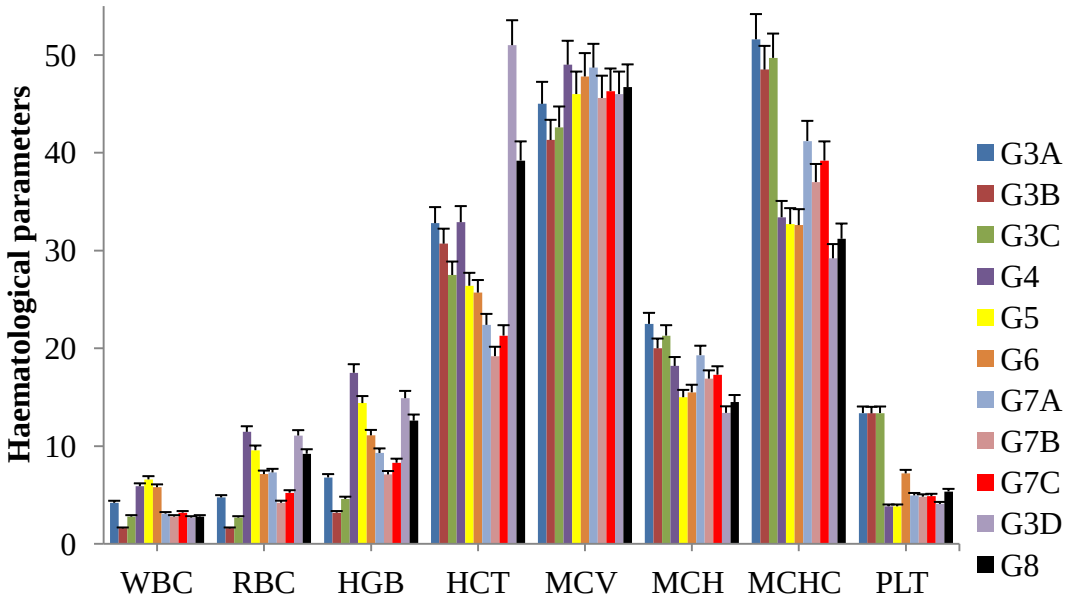

Fig. 10. Effects of combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio 1:1 on haematological parameters of albino mice infected with *E. coli, Salmonella* and *Shigella* respectively

KEY: G-GROUP
Group 3A, 3B & 3C *were infected with E. coli, Salmonella and Shigella respectively and treated with extract (70.71mg/ml/body weight (kg)*
Group 3D *were not infected but fed with extract (70.71mg/ml/body weight (kg)*
Groups 4, 5 & 6 *were infected with E. coli, Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*
Group 7A, 7B & 7C *were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.*
Group 8 *were neither infected nor treated (they received distilled water during the treatment period)*
WBC*-White blood cell (/µL×10^3),* ***RBC****- Red blood cell count(/µ×10^6),* ***HGB-****Haemoglobin concentration (g/dL),* ***HCT****-Hematocrit packed cell volume (%),* ***MCV-****Mean corpuscular volume (fL),* ***MCH****-Mean corpuscular hemoglobin (Pg),* ***MCHC****- Mean corpuscular hemoglobin concentration (g/dL),* ***PLT****-Platelet (/µL×10^5)*

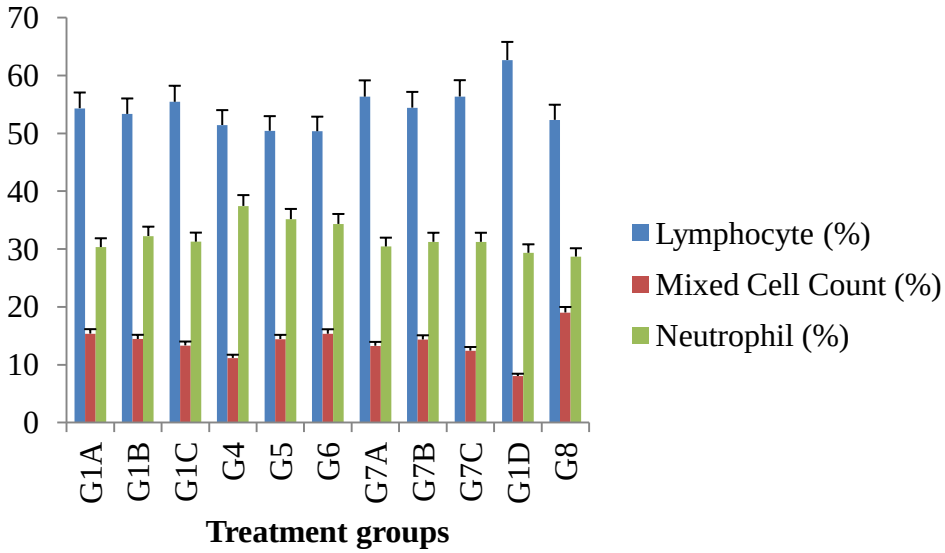

Fig. 11. Effects of water extract of *A. sativum* on Differential White Blood Cell counts of albino mice infected with *E. coli, S. typhi* and *S.dysenteriae* respectively

KEY: G-GROUP

Group 1A, 1B & 1C *were infected with E. coli, Salmonella and Shigella respectively and treated with extract (223.61mg/ml/body weight (kg)*

Group 1D *were not infected but fed with extract (223.61mg/ml/body weight (kg)*

Groups 4, 5 & 6 *were infected with E. coli, Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*

Group 7A, 7B & 7C *were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups.*

Group 8 *were neither infected nor treated (they received distilled water during the treatment period)*

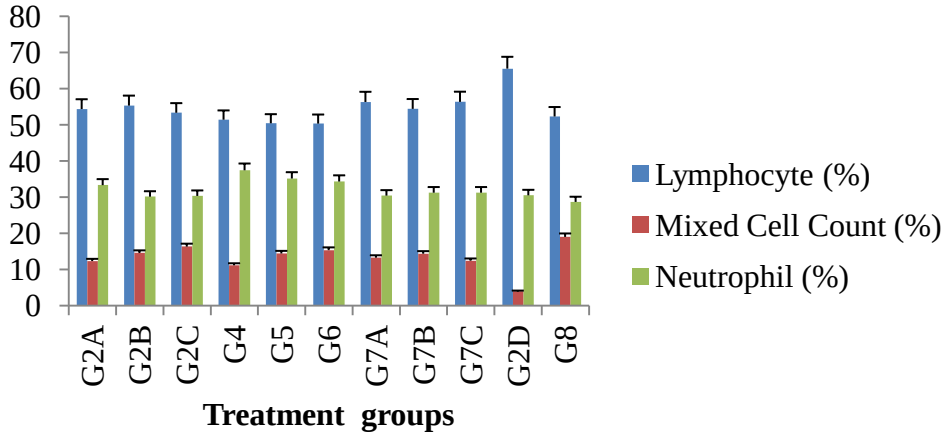

Fig. 12. Effects of ethanol extract of *N. lotus* Root on Differential White Blood Cell counts of albino mice infected with *E. coli, Salmonella* and *Shigella* respectively

KEY: G-GROUP

Group 2A, 2B & 2C *were infected with E. coli, Salmonella and Shigella respectively and treated with extract (70.71mg/ml/body weight (kg)*

Group 2D *were not infected but fed with extract (70.71mg/ml/body weight (kg)*

Groups 4, 5 & 6 *were infected with E. coli, Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*

Group 7A, 7B & 7C *were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups*

Group 8 *were neither infected nor treated (they received distilled water during the treatment period)*

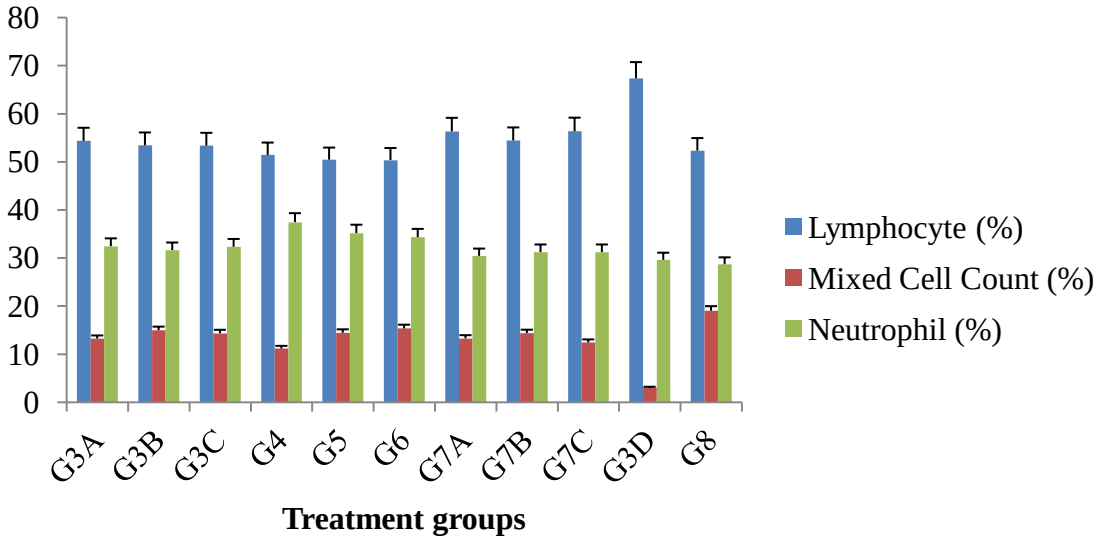

Fig. 13. Effects of combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio 1:1 on differential white blood cell counts of albino mice infected with *E. coli, Salmonella* and *Shigella* respectively

KEY: G-GROUP
Group 3A, 3B & 3C *were infected with E. coli, Salmonella and Shigella respectively and treated with extract (70.71mg/ml/body weight (kg)*
Group 3D *were not infected but fed with extract (70.71mg/ml/body weight (kg)*
Groups 4, 5 & 6 *were infected with E. coli, Salmonella and Shigella respectively but not treated (received sterile distilled water during the treatment period) negative control group*
Group 7A, 7B & 7C *were infected with E. coli, Salmonella and Shigella respectively and treated with ciprofloxacin (2 mg/ml/body weight (kg) positive control groups*
Group 8 *were neither infected nor treated (they received distilled water during the treatment period)*

4. DISCUSSION

4.1 Sensitivity Pattern of Bacterial Isolates to Extracts of *N. lotus* and *A. sativum*

Antimicrobial susceptibility patterns of the combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root inhibit all the bacteria at a lower concentration. This may be due to the presence of active antimicrobial phytochemical compound present in the two extracts [18]. *N. lotus* and *A. sativum* extracts had a lower Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) to the isolated bacteria which indicates these extracts can inhibit the growth of enteric bacteria at a lower concentration. This is related to Akinjogunla et al. [19] findings in which they reported a lower MIC and MBC of *N. lotus* extract at 10-30 mg/ml against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* respectively. The interaction of ethanol extract of *N. lotus* root in combination with water extract of *A. sativum* as antimicrobial against resistant enteric bacteria at ratio 1:1 reveals that the combination of the two extracts at ratio 1:1 was indifferent for all enteric bacteria. This is in contrast with Mohammad and Rawaa [20] findings of synergistic effect of *Thymbra spicat* L. extracts with antibiotics against multidrug- resistant *Staphylococcus aureus* and *Klebsiella pneumonia* strains in which there were synergistic interaction between *Thymbra spicata* extracts and commercially available antibiotics against the bacteria.

4.2 Toxicity Effect *A. sativum* and *N. lotus* Extracts on Swiss Albino Mice

The toxicity effect of water extract of *A. sativum* reveals that *A. sativum* was nontoxic to the experimental mice, the mice were hyperactive and anorexia was observed and there was no mortality during the seven days acute toxicity assay which is accordance with Lawal et al. [21] findings in which they claim there were no toxic effect of *A. sativum* at concentration 300, 600

and 1200 mg/ml/kg on the experimental rat's liver and kidney tubules. The toxicity effect of ethanol extract of *N. lotus* root on albino mice shows that the mice were very active and there was no sign of toxicity observed at lower concentration in the experimental mice. This is related to the findings of Sharaibi et al. [22] in which they observe that a single oral administration of 5000 mg/kg body weight aqueous extract of *N. lotus* leaves did not cause any mortality or alteration in behavioural or physiological state of rats in their study. This is an indication that *N. lotus* root extract is not harmful at lower concentration. However, at high concentrations of the extracts most of the experimental mice were not active and anorexia was observed. It was also observed that there was no sign of toxicity in the experimental mice tested for toxicity effect of water extract of *A. sativum* and ethanol extract of *N. lotus* root in combination at ratio (1:1). There was no mortality during the seven days toxicity assay.

4.3 Body Weight Gain Trend for Swiss Albino Mice Treated with Water Extract of *Allium sativum*, Ethanol Extract of *N. lotus* Root and Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio (1:1)

There was significant ($p<0.05$) weight gain in experimental mice during toxicity test and during treatment of experimental mice after infection for extracts of water extract of *Allium sativum*, ethanol extract of *N. lotus* root and combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio (1:1) respectively with extract of combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio (1:1) having the highest weight gain at lower concentration of the extracts. This may be attributed to nutrients components that may be present in there feed and plants extracts. This is in contrast with the findings of Mak-Soon et al. [23] which they reported a reduction in weight of *A. sativum* diet-Induced obese mice, and agrees with Sharaibi et al. [22] findings in which they reported a significant increase in the body weight of experimental rat treated with *N. lotus* leaves extracts.

4.4 Change in Body Temperature (^oC) of Experimental Animals during Treatment

There was significant ($p<0.05$) decrease in body temperature of experimental mice at body weight of all extracts during toxicity test and during treatment of experimental mice after infection. This may be attributed to nutrients components that have the ability to slow down the rate of heart beat that may be present in there feeds and plants extracts. This is in accordance with the findings of Mak-Soon *et al.* [23] which they reported a reduction in temperatures of *A. sativum* diet-Induced obese mice.

4.5 Effects of Treatment with Water Extract of *Allium sativum*, Ethanol Extract of *N. lotus* Root and Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio (1:1) on Faecal Shedding of *Escherichia coli*, *Salmonella* and *Shigella* (CFU/g)

The effect of treatment with *A. sativum* and *N. lotus* extracts on faecal shedding of *Escherichia coli*, *Salmonella* and *Shigella* in which there was decrease in number of viable colony of the test bacterial isolate which is in accordance with findings of results of Donald et al. [24] in which they reported that the administration of aqueous extract of *Euphorbia prostrata* inhibited the growth of *Salmonella typhimurium*, and thus reduced the numbers of viable *Salmonella typhimurium* recovered from faeces.

4.6 Effects of Water Extract of *Allium sativum*, Ethanol Extract of *N. lotus* Root and Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio (1:1) Extracts on Haematological Parameters of Swiss Albino Mice Infected with *Escherichia coli*, *Salmonella* and *Shigella*

Effects of water extract of *Allium sativum*, ethanol extract of *N. lotus* root and combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio (1:1) extracts on hematological parameters of Swiss Albino mice infected with *Escherichia coli*, *Salmonella* and *Shigella* revealed that the low hematocrit of Group 7B (infected with *Salmonella* and treated with ciprofloxacin) may be due to mechanism action of the antibiotics. This was in accordance with findings of Kalpana et al. [25]. In which ciprofloxacin brought a decrease in RBC, WBC, Hb and blood glucose level in a dose dependent manner.

4.7 Effects of Extracts of *A. sativum* and *N. lotus* on Differential White Blood Cell Counts of Albino Mice Infected with *E. coli, Salmonella* and *Shigella* Respectively

The effects of water extract of *Allium sativum*, ethanol extract of *N. lotus* root and combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio (1:1) extracts on differential white blood cell counts of albino mice infected with *E. coli, Salmonella* and *Shigella* respectively reveals that group 6 (were infected with *Shigella* but not treated, received sterile distilled water during the treatment period) has the lowest lymphocyte cell counts for all groups which was below the normal range (65 – 87 %) of mice lymphocyte cell counts. This may be due to shock from *Shigella* toxins while group 4 (were infected with *E. coli* but not treated (received sterile distilled water during the treatment period) had the highest percentage neutrophil, which may be triggered due to immunological response of the mice to the infection. Group 8 (were neither infected nor treated (they received distilled water during the treatment period) had the least percentage neutrophil which was above the normal range of mice neutrophil (7.5 – 27 %). This was similar to Stephanie et al. [26] findings in which Antigen-Specific $CD8^+$ T Cells fail to respond to *Shigella flexneri*.

4.8 Histological Effects of Treatment with Water Extract of *A. sativum*, Ethanol Extract of *N. lotus* Root and Combination of Water Extract of *A. sativum* and Ethanol Extract of *N. lotus* Root at Ratio 1:1 on Organs Invasion of *Salmonella* as a Representative for Other Enteric Bacteria

The histological effects of treatment with water extract of *A. sativum*, ethanol extract of *N. lotus* root and combination of water extract of *A. sativum* and ethanol extract of *N. lotus* root at ratio 1:1 on organs invasion of *Salmonella* indicates that major pathological effects were exerted by the treatment on the liver and kidney of the experimental mice compared with liver and kidney pathological features of the control groups. This observation is related to Khalid et al. [17] findings in which the liver, kidney, and spleen of mice exposed to gold nanoparticles had pathological changes while the liver, kidney, and spleen of mice that were not exposed to gold nanoparticles did not have pathological changes.

5. CONCLUSION

This study reveals the combination of extracts of *N. lotus* and *A. sativum* were able to inhibit multi-drug resistant enteric bacteria at a lower concentration effectively. The combination of this extracts recorded a low Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against the resistant enteric bacterial isolates. The interaction of ethanol extract of *N. lotus* root in combination with water extract of *A. sativum* as antimicrobial against isolated enteric bacteria at ratio 1:1 reveals that the combination of the two extracts at ratio 1:1 was indifferent for all enteric bacteria.

There was no sign of toxicity observed for water extract of *A. sativum* and when combined with ethanol extract of *N. lotus* root at ratio (1:1) at concentration of 1000mg/ml/kg body weight of experimental mice which makes it safe for the vital organs in the body at that concentration. Ethanol extract of *N. lotus* root showed a sign of toxicity at concentration of 500 & 1000mg/ml/kg body weight of experimental mice. This study reveals that ciprofloxacin had effects on the hematocrit level of the mice thereby causing reduction in their hematocrit level. Low lymphocyte and high neutrophil were recorded for test groups that infected and not treated. Histopathological assay of this study reveals that infection and treatment had histopathological effects on the liver and kidney of the experimental mice.

6. RECOMMENDATION

Extensive *In vivo* trials should be done to determine the immunomodulatory effects of these extracts.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/70491