

## Original Research Article

### Immunomodulatory and Antibacterial Effects of Honey in Wistar rats infected with *Salmonella typhimurium*.

#### ABSTRACT

**Aims:** To evaluate the immunomodulatory and antibacterial effects of honey on Wistar rats infected with *Salmonella typhimurium*.

**Place and Duration of Study:** Research laboratory of Federal University of Technology Akure (FUTA), Ondo State, Nigeria between July 2019 to September 2019.

**Methodology:** A total of thirty – nine (39) apparently healthy Wistar rats of three (3) rats per group were used in this study. 12 out of the rats was used to determine infectivity dose and twenty – seven (27) for infection and treatment assay. The rats were divided into nine (9) groups of 3 rats per group, all the groups were infected with *S. typhimurium* and treated for seven (7) days with honey, augmentin and oral rehydration salt (ORS) except group 1 that was infected and not treated and group 9, not infected, not treated. The blood samples of all the rats was collected after treatment to study the effect of honey on the haematological parameters of the rats.

**Results:** All the honey samples used exerted growth inhibitory activity on *Salmonella typhimurium* but honey from FUNAAB worked best both in the *in-vivo* and *in-vitro* studies. The rats that were infected with the *S. typhimurium* and treated with 2ml and 3ml honey gave a good therapeutic potential in combating diarrhoea in the animals. Also, in these groups of rats, honey caused an increase in the PCV, RBC, HB and lymphocytes which displays honey to be a good immunostimulator and immunomodulator.

**Conclusion:** This honey has also exerted antibacterial, haematinic and immunomodulatory potentials when rats infected with *S. typhimurium*. These findings, therefore could be exploited in the treatment of diarrhoeal diseases caused by this bacterium.

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**Keywords:** [*S. typhimurium*, Wistar rats, Honey, Augmentin, Oral rehydration salt (ORS), Immunomodulation]

## 1. INTRODUCTION

*Salmonella typhimurium* are Gram-negative, flagellated, aerobic (oxygen-consuming) bacteria that are the major cause of human salmonellosis [1], a type of gastroenteritis, or inflammation of the intestine [2]. *S. typhimurium* is also a frequent cause of acute, self-limiting food borne diarrhea, it is spread primarily by contaminated food and drink, but it can come in contact with a human via direct contact with an infected animal or pet [1]. *Salmonella typhimurium* induces a systemic infection in rats, So *S. typhimurium*-infected rats have been extensively used as models for the understanding of the immunological and antibacterial effect of honey. Diarrhoeal diseases are among the leading causes of morbidity and mortality in young children in developing countries [3] It is characterized by frequent, loose and watery stool which may result in dehydration and in severe cases, death. Each year , an estimated 2.5 billion cases of diarrhoea occur among the children under five years of age, and estimates suggest that overall incidence has remained relatively stable over the past two decades. When this illness is as a result of *S. typhimurium* infections when antibiotics therapy may be required, the problem of antibiotics resistance is also a serious problem because almost all known strains of *S. typhimurium* have developed resistant to most of the commonly employed antibiotics Also, some of these antibiotics can induce diarrhoea known as “antibiotic induced diarrhoea”[4]. Therefore, it becomes imperative to search for alternatives to conventional antibiotics to treat this disease. In most ancient cultures, honey has been used for both nutritional and medical purposes. The belief that honey is a nutrient, a drug and an ointment has been carried into our days, and thus, an alternative medicine branch, called “apitherapy”, has been developed in recent years, offering treatments based on honey and other bee products against many diseases including bacterial infections. Honey has been reported to have immunomodulatory and antibacterial activity on bacteria found in wounds [5], responsible for food spoilage[6], common diarrhoeagenic bacteria such as *S. typhimurium* [7] and many other bacterial species.

## 2. MATERIAL AND METHODS

### 2.1 Location and Duration of the Research

The research was carried out in the Graduate Research Laboratory of Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria between July to September, 2019.

### 2.2 Collection of Honey Samples

Honey samples were collected from ten (12) different locations in Nigeria; Emure – Ile and Afo – Akoko, Ikakuma – Akoko, Akure in Ondo State, Enugu, Enugu State, Ibadan, Oyo State, Ikere- Ekiti, Ekiti State, Lagos, Lagos State, Nasarawa, Nasarawa State, FUNAAB, Abeokuta Ogun State, Zamfara, Zamfara State and Iree, Osun State. Table 1 shows the location and the floral source of the honey samples used.

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2 **Table 1. Honey samples from different locations in Nigeria**

S/N	Location	Floral source
1	Emure – Ile, Ondo State (Roadside)	Wildflower Honey
2	Ikere- Ekiti, Ekiti State	Wildflower Honey
3	Nasarawa, Nasarawa State	Wildflower Honey
4	Ibadan, Oyo State	Wildflower Honey
5	Afo, Ondo State	Wildflower honey
6	Ire, Osun State	Bitter leaf
7	FUNAAB, Abeokuta, Ogun State	Wildflower Honey
8	Enugu, Enugu State (Cinomis Honey)	Wildflower Honey
9	Lagos, Lagos State (Kaybeck Honey)	Wildflower Honey
10	Zamfara, Zamfara State (A & Shine Honey)	Wildflower Honey
11	Ikakuma – Akoko, Ondo State	Wildflower Honey
12	Akure, Ondo State	Wildflower Honey

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### 5 **2.3 Test Organism**

6 The test organism used was *Salmonella typhimurium*

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#### 8 **2.3.1 Isolation and identification of the test organism**

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10 *S. typhimurium* was isolated from the faeces of poultry droppings, the stool sample was  
 11 serially diluted in sterile distilled water using the method of [8]. The dilutions were plated  
 12 on Salmonella Shigella agar to isolate the bacteria and was identified based on  
 13 morphological and biochemical characteristics according to the method of [9].

### 14 **2.4 Antibacterial assay**

15 The antibacterial activities of all the honey samples (unpasteurised and  
 16 pasteurised) on the *S. typhimurium* was done using agar well diffusion  
 17 method according to [10].

### 18 **2.5 Experimental animals**

19 A total of 39 female Wistar rats of 60-90g were used for the study. The animals were  
 20 purchased at Animal Production and Health Dept of Federal University of Technology  
 21 Akure, Ondo State. They were brought to animal house of Microbiology department,  
 22 FUTA and acclimatized for 7days before the commencement of this work. The animals  
 23 were fed with broiler starter and clean water twice daily.

### 24 **2.6 Experimental animals for infectivity dose**

25 A total of 12 rats female apparently Wistar rats of 60-90g was used to determine  
 26 infectivity dose. The rats were divided into four groups of 3 rats per cage.

27 **2.6 Determination of infectivity dose (ID) of *Salmonella typhimurium***

28 This was done using standard method described by [11].

29 A colony of *S. typhimurium* of 24hrs old was inoculated into 100ml of Nutrient agar,  
30 incubated at 37°C for 18 – 24hrs. The cells were harvested by centrifuging at 3000rpm  
31 for 15 minutes. The supernatant was decanted and 10ml of sterile normal saline was  
32 poured into the tube and was further centrifuged to wash the cells, this was done three  
33 times. Serial dilution was carried on the harvested cells. 1ml was taken from each of the  
34 different concentrations already prepared to infect the experimental animals. The dilution  
35 that produced the symptoms of illness in all of the animals was taken as the infectivity  
36 dose (ID) of the organism.

37 **2.7 Experimental design**

38 A total of 27 female apparently healthy Wistar rats were assigned into nine (9)  
39 treatment groups designated as 1 – 9. i.e. 3 rats per cage. Rats in group 1 were  
40 infected with the ID of *S. typhimurium* and not treated, group 2 were infected and  
41 treated with 1ml raw honey 12hourly, group 3 infected and treated with 2ml raw honey  
42 12hourly, group 4 infected and treated with 3ml raw honey 12hourly, group 5 infected  
43 and treated with 0.5ml Augmentin (30mg/kg/day) 12hourly, group 6 infected and  
44 administered honey – ORS 12hourly, group 7 infected and administered 1ml  
45 commercial ORS 12hourly, group 8 infected and administered 1ml homemade ORS  
46 12hourly and group 9 not infected, not treated (control group).

47 **2.8 Infection of rats with *S. typhimurium***

48 The infection of the animals was done using the infectivity dose of the  
49 organism by orogastrically dosing them according to the method of [11]. The  
50 infectivity dose used in this study was calculated to be  $1.5 \times 10^8$  cfu/ml.

51 **2.9 Treatment of infected rats**

52 Treatment begins 24hours after which infection has set in, specific volume of  
53 honey, augmentin, honey –ORS, ORS both commercial and home made  
54 variant were administered to the infected rats for 7 days according to [12].

55 **2.10 Isolation, identification and enumeration of *S. typhimurium* in the faeces of**  
56 **infected rats**

57 1g of faeces of the infected rats were aseptically collected, serial dilution was done on them  
58 and plated on salmonella shigella agar in order to isolate the *Salmonella typhimurium* present  
59 in the rats and monitor their bacterial count through out the experiment.[13].

60 **2.11 Weighing of Animals**

61 The weight of the animals were taken throughout the pre and post ingestion  
62 period using the method of [14].

### 63 **2.12 Haematological Assay**

64 The blood of apparently healthy Wistar albino rats was collected weekly into EDTA  
65 bottles after which the Packed Cell Volume (PCV), Haemoglobin (HB), Red blood Cell  
66 (RBC), White Blood Cell (WBC) and differential leukocytes counts of the collected  
67 blood samples were evaluated according to the method described by [16].

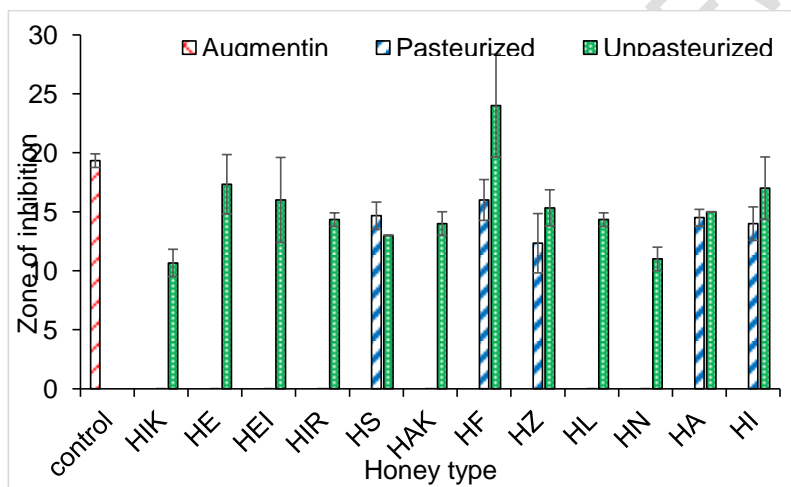
### 68 **2.13 Statistical Analysis**

69 All experiments were done in triplicates, Mean, Standard deviation were calculated for  
70 all data using Descriptive Statistics and difference between means was determined  
71 by Duncan's New Multiple Range Test at  $p \leq .05$ .

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73 **3. RESULTS AND DISCUSSION**

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 75 Almost all the honey samples used in this study inhibited the growth of *S. typhimurium*,  
 76 the antibacterial activity exerted by honey on the test organism in this study is in  
 77 agreement with the work carried out by [7] that pure honey has bactericidal activity  
 78 against *Salmonella* species. There was a great reduction in the antibacterial activities  
 79 of pasteurised honey when compared with the unpasteurised ones, this is in  
 80 agreement with the work of [16] and [17] that reported the loss of antibacterial activity  
 81 on exposure of honey to heat at 80°C for 10mins or 56°C for 30mins. Honey from  
 82 FUNAAB was observed to have the highest antibacterial activity when compared with  
 83 the control (augmentin) used in this study. (Fig.1).



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 87 Fig. 1: Comparison among the antibacterial activities of unpasteurised and pasteurised honey samples  
 88 on *S. typhimurium* isolated from poultry droppings.

89 Key: HIA = Honey from Ikakuma- Akoko, HIK = Honey from Ikere – Ekiti, HN  
 90 = Honey from Nasarawa, HI = Honey from Ibadan, HA = Honey from Afo –  
 91 Akoko, HIR = Honey from Iree, HF = Honey from FUNAAB, HE = Honey from  
 92 Enugu, HL = Honey from Lagos, HZ = Honey from Zamfara, HS = Sunshine  
 93 honey and HEI = Honey from Emure – Ile.

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 96 All the rats infected with this bacterium and treated with honey recovered by the 3<sup>rd</sup> day  
 97 while those ones that was administered honey – ORS and the ones with homemade ORS  
 98 recovered by day 4 and those treated with augmentin and those administered commercial  
 99 ORS recovered by the 5<sup>th</sup> day. those that were infected but not started showing signs of  
 100 recovery by the 6<sup>th</sup> day, this could be that acute diarrhoea is self limiting according to [18].  
 101 The mean recovery times of rats infected with *Salmonella typhimurium* and treated with honey

102 –ORS was significantly reduced when compared with infected and not treated group, this is  
 103 agreement with the work of [19] (Table 2). There was no evidence of *S. typhimurium* in the  
 104 faeces of rats treated with 1ml, 2ml, 3ml of honey and administered 1ml of honey –ORS  
 105 12hourly for 7 days. (Fig. 2).  
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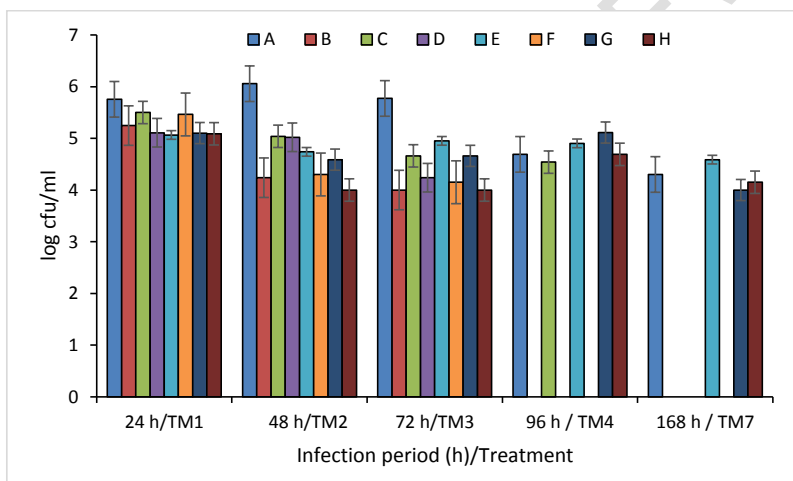
110 Table 2: Physical observations of the Wistar rats during infection with *Salmonella typhimurium*  
 111 and treatment

Group of rats	Treatment	(Days)						
		1	2	Interval 3	4	5	6	7
1	Infected and not treated with honey	RA, EL, UF,PM,SS	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	A, EW, FS, NM, SF	A, EW, FS, NM, SF
2	Infected and treated with 1ml honey (12hourly)	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	A, EW, FS, NM	A, EW, FS, NM	A, EW, FS, NM	A, EW, FS, NM	A, EW, FS, NM
3	Infected and treated with 2ml honey (12hourly)	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	A, EL, UF, PM, SS	A, EL, FS, NM, SF	A, EL, FS, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF
4	Infected and treated with 3ml honey (12hourly)	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	A, EL, UF, PM, SS	A, EL, FS, NM, SF	A, EL, UF, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF
5	Infected and treated with 0.5ml Augmentin (12hourly)	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	A, EW, FS, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF
6	Infected and treated with 1ml Honey - ORS (12hourly)	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	A, EW, FS, NM, SF	A, EW, FS, NM	A, EW, FS, NM, SF	A, EW, FS, NM, SF
7	Infected and treated with 1ml ORS (12hourly)	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	RA, EL, UF, *PM, SS	A, EW, FS, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF
8	Infected and treated with	RA, EL, UF, PM,	RA, EL, UF,	RA, EL, UF, PM,	A, EW, FS, *PM,	A, EW, FS, NM,	A, EW, FS, NM,	A, EW, FS, NM,

	1ml homemade (12hourly)	SS	PM, SS	SS	SS	SF	SF	SF
9	Not infected and not treated	A, EW, FS, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF

112 **KEY:** A = Activeness, RA = Reduced activity, EL = Eating little, EW = Eating  
 113 well, UF = informed stool, FS = Formed stool, SF = Smooth fur, SS = Scattered  
 114 fur, PM = Presence of mucous, \*PM = High presence of mucous, NM = No  
 115 mucous

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 117 There was no evidence of *Salmonella typhimurium* in the faeces of rats treated with 1ml,  
 118 2ml, 3ml of honey and administered 1ml of honey –ORS 12hourly for 7 days. (Fig. 2).  
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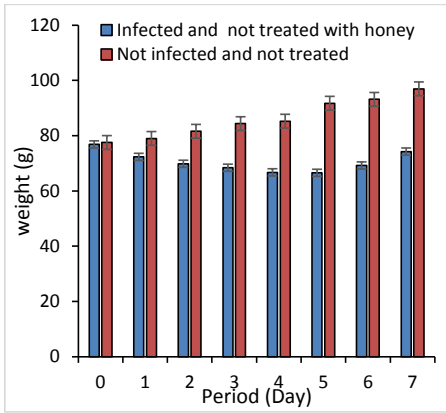


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 123 Fig. 2: Total counts of *S. typhimurium* in the faeces of Wistar faeces after infection and  
 124 treatment

125 **KEY:** A = Infected and not treated, B = Infected and treated with 1ml honey, C = Infected and treated  
 126 with 2ml honey, D = Infected and treated with 3ml honey, E = Infected and treated with 0.5ml  
 127 augmentin, F = Infected and treated with 1ml honey – ORS, G = Infected and treated with 1ml  
 128 commercial ORS, H = Infected and treated with 1ml homemade ORS, TM1 = Treatment day 1, TM2 =  
 129 Treatment day 2. TM3 = Treatment day 3, TM4 = Treatment day 4 and TM7 = Treatment after day 7.  
 130 The observation that the infected and not treated rats did not gain back their  
 131 body weight throughout the duration of the research is in agreement with the  
 132 work of [14]. (Fig. 3)..  
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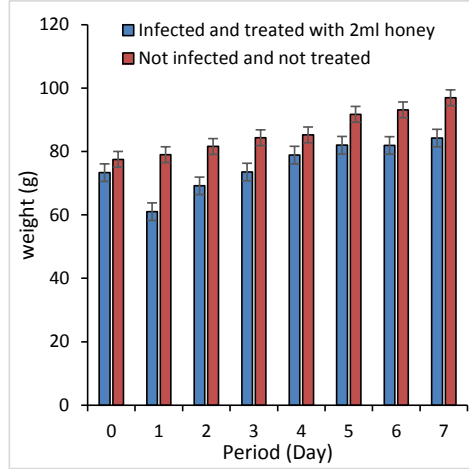


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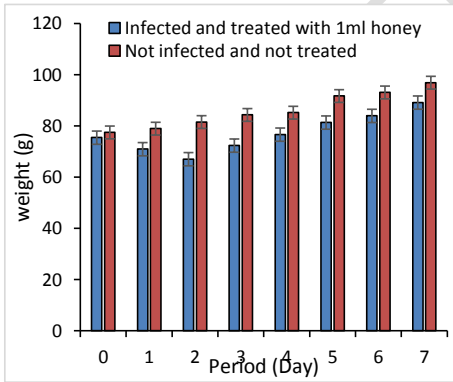
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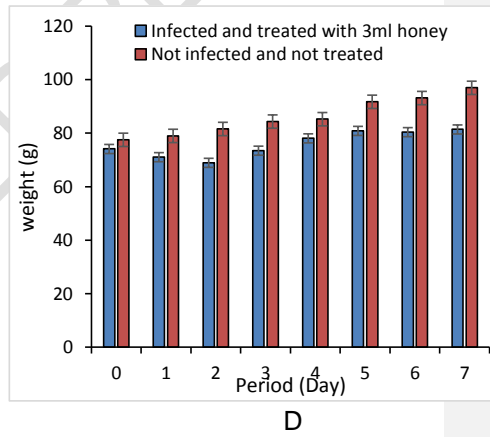


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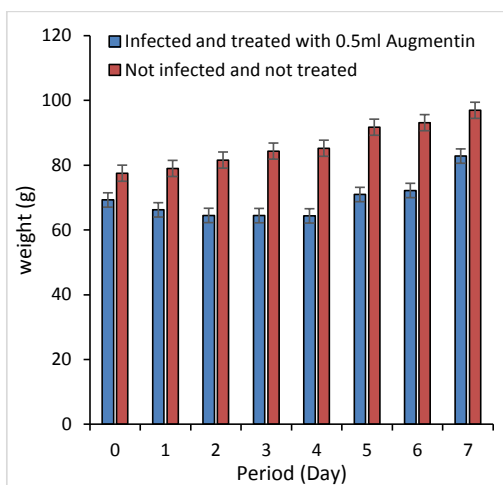
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**D**

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Fig, 3A –E : Average weight of Wistar rats before infection with *S. typhimurium*, at the onset of infection and after treatment with honey and augmentin.

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Infection of rats in group 1 that were not treated caused a decrease in their PCV, HB and RBC, their neutrophils was so high, showing a sign of infection, there was no significant difference in the PCV, WBC of the group of rats treated with 2ml, 3ml of honey and the group not infected, not treated (control) ( Table 3a and 3b). Administration of honey to apparently healthy rats (control) and the groups infected with *S. typhimurium* and treated with honey caused a significant ( $p < .05$ ) increase in the PCV and lymphocytes of the rats, this shows that the honey has haematonic and immunomodulatory potentials. (Table 3a and 3b).

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184 Table 3a: Effect of honey on the haematological parameters of Wistar rats  
 185 infected with *S. typhimurium*  
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Group	PCV (%)	HB (g/L)	WBC (10 <sup>9</sup> /L)	RBC (10 <sup>12</sup> /g/L)
1	32.50 ± 4.95 <sup>b</sup>	10.85 ± 1.66 <sup>b</sup>	12.70 ± 0.28 <sup>a</sup>	3.00 ± 0.82 <sup>b</sup>
2	40.00 ± 2.83 <sup>ab</sup>	13.50 ± 0.71 <sup>ab</sup>	11.70 ± 0.28 <sup>ab</sup>	3.20 ± 0.25 <sup>ab</sup>
3	42.50 ± 2.12 <sup>a</sup>	14.14 ± 0.66 <sup>a</sup>	8.77 ± 0.49 <sup>c</sup>	4.04 ± 0.87 <sup>ab</sup>
4	43.00 ± 4.24 <sup>a</sup>	14.30 ± 1.36 <sup>a</sup>	8.94 ± 0.42 <sup>c</sup>	4.29 ± 0.07 <sup>a</sup>
5	40.33 ± 4.51 <sup>ab</sup>	13.50 ± 1.51 <sup>ab</sup>	10.86 ± 0.17 <sup>abc</sup>	4.06 ± 0.30 <sup>ab</sup>
6	35.50 ± 0.71 <sup>ab</sup>	11.90 ± 0.28 <sup>ab</sup>	10.88 ± 0.05 <sup>abc</sup>	3.66 ± 0.03 <sup>ab</sup>
7	39.67 ± 4.51 <sup>ab</sup>	13.19 ± 1.56 <sup>ab</sup>	9.56 ± 1.92 <sup>bc</sup>	4.07 ± 0.34 <sup>ab</sup>
8	35.00 ± 6.00 <sup>ab</sup>	11.64 ± 2.00 <sup>ab</sup>	9.83 ± 1.47 <sup>bc</sup>	3.66 ± 0.53 <sup>ab</sup>
9	42.33 ± 2.08 <sup>a</sup>	13.66 ± 0.35 <sup>ab</sup>	8.82 ± 1.06 <sup>c</sup>	4.07 ± 0.58 <sup>ab</sup>

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 188 Table 3b: Effect of honey on the haematological parameters of Wistar rats infected with *S.*  
 189 *typhimurium* contd  
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Group	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	31.50 ± 0.71 <sup>f</sup>	64.50 ± 0.71 <sup>a</sup>	3.00 ± 0.00 <sup>a</sup>	1.50 ± 0.71 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>
2	37.50 ± 0.71 <sup>bc</sup>	61.00 ± 1.41 <sup>ab</sup>	2.50 ± 0.71 <sup>a</sup>	2.00 ± 1.41 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>
3	40.00 ± 2.83 <sup>ab</sup>	56.50 ± 0.71 <sup>b</sup>	1.50 ± 0.71 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00
4	40.50 ± 0.71 <sup>a</sup>	57.00 ± 1.41 <sup>b</sup>	1.50 ± 0.71 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00
5	39.33 ± 150 <sup>abc</sup>	56.00 ± 4.00 <sup>b</sup>	1.67 ± 1.15 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00
6	37.00 ± 1.41 <sup>cd</sup>	60.00 ± 2.83 <sup>ab</sup>	2.00 ± 1.41 <sup>a</sup>	1.50 ± 0.71 <sup>a</sup>	0.00 ± 0.00
7	32.67 ± 1.15 <sup>ef</sup>	60.33 ± 3.79 <sup>ab</sup>	2.00 ± 1.00 <sup>a</sup>	1.67 ± 0.58 <sup>a</sup>	0.00 ± 0.00
8	34.67 ± 0.58 <sup>de</sup>	60.67 ± 1.15 <sup>ab</sup>	2.33 ± 0.58 <sup>a</sup>	1.67 ± 0.58 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>
9	40.00 ± 1.00 <sup>ab</sup>	57.33 ± 1.15 <sup>b</sup>	1.33 ± 0.58 <sup>a</sup>	1.33 ± 0.58 <sup>a</sup>	0.00 ± 0.00

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#### 4. CONCLUSION

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This study has shown that the antibacterial activity of honey sample from FUNAAB (HF) against *S. typhimurium* as the most effective in inhibiting the growth of the organism. This honey has also exerted antibacterial, haematinic and immunomodulatory potentials when rats infected with *S. typhimurium*. These findings therefore could be exploited in the treatment of diarrhoeal diseases caused by this bacterium.

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#### 207 **COMPETING INTERESTS**

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209 Authors have declared that no competing interests exist.

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#### 212 **CONSENT**

213 It is not applicable

214

#### 215 **ETHICAL APPROVAL**

216 As per international standard written ethical approval has been collected and

217 preserved by the author(s).

218

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