Original Research Article

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

.

ABSTRACT

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

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

Methodology: A total thirty – nine (39) apparently healthy Wistar rats, three (3) rats per group were used in this study. Twelve (12) out of the rats were used to determine the infectivity dose of *S*. *typhimurium* on the rats and twenty – seven (27) rats for infection and treatment assay. The rats were divided into nine (9) groups of 3 rats per group, the first 8 groups were infected with *S*. *typhimurium* and treated for seven (7) days with honey, augmentin and oral rehydration solution (ORS) (different treatment for different groups) except group 1 that was infected and not treated and group 9, that was 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: Honey administered at 2ml and 3ml twice daily to the *S*. *typhimurium* infected rats exerted good therapeutic potential in combating diarrhoea in the animals. Also, in these group of rats, honey caused an increase in the PCV, RBC, HB and lymphocytes which displays honey to be a good immunostimulator and immunomodulator.

Conclusion: Honey exerted therapeutic, haematinic and immunomodulatory potentials in rats infected with *S. typhimurium*. These findings therefore could be exploited in the treatment of diarrhoeal diseases caused by this bacterium.

Keywords: [S. typhimurium, Wistar rats, Honey, Augmentin, Oral rehydration solution (ORS), Immnuomodulation]

1. INTRODUCTION

Salmonella typhimurium is a Gram-negative, flagellated, aerobic (oxygen-consuming) bacteriium, 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 diarrhoea. It is spread primarily by contaminated food and drink, but it can come in contact with 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. Although, diarrhoea is self-limiting however when it is as a result of bacterial infection, antibiotic therapy might be required but because of the high resistance rate of bacteria to available antibiotics, administration of antibiotics may not result in recovery of patients. Moreover, some of these antibiotics can also 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. It becomes worthwhile therefore to investigate whether honey has therapeutic and immunostimulatory potentials in Wistar rats infected with *S. typhimurium* in addition to nits antibacterial potential.

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 and September, 2019.

2.2 Honey Sample

The honey sample used was collected from FUNAAB, Abeokuta Ogun State. It’s of wild flora source.

2.3 Test Organism

The test organism used was *Salmonella typhimurium*

2.3.1 Isolation and identification of the test organism

S. typhimurium was isolated from poultry droppings, the droppings was serially diluted in sterile distilled water using the method of Boateng and Diunase [8]. The dilutions were plated on Salmonella - Shigella agar to isolate the bacterium and was identified based on morphological and biochemical characteristics according to the method of Rozanska [9].

2.4 Experimental animals

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

2.5 Determination of infectivity dose (ID) of *Salmonella typhimurium*

A total of twelve (12) female apparently Wistar rats was used to determine infectivity dose. The rats were divided into four groups of 3 rats per cage. This was done using standard method described by Adebolu *et al.* [10]. A colony of *S. typhimurium* of 24hrs old was inoculated into 100ml of Nutrient agar, incubated at 37^{o}C for 18 – 24hrs. The cells were harvested by centrifuging at 3000rpm for 15 minutes. The supernatant was decanted and 10ml of sterile normal saline was poured into the tube and was further centrifuged to wash the cells, this was done three times. Serial dilution was carried on the harvested cells and 1ml was taken from each of the different concentrations already prepared to infect different groups of the experimental animals respectively. The dilution that produced the symptoms of illness in all of the animals was taken as the infectivity dose (ID) of the organism.

2.6 Experimental design

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

2.7 Infection of rats with the ID of *S. typhimurium*

The infection of the animals was done using the infectivity dose (ID) of the organism by orogastrically dosing them according to the method of Adebolu

et al. [10]. The infectivity dose used in this study was calculated to be 1.5 x10^8 cfu/ml.

2.8 Treatment of infected rats

Treatment begins 24hours after which infection has set in, specific volume of honey, augmentin, honey –ORS, ORS both commercial and home - made variants were administered to the infected rats for 7 days according to Oladunmoye [11].

2.9 Isolation, identification and enumeration of *S. typhimurium* in the faeces of infected rats

One gram (1g) of faeces of the infected rats were aseptically collected, serial dilution was done on them and plated on salmonella shigella agar in order to isolate the *Salmonella typhimurium* present in the rats and monitor their bacterial count throughout the experiment [12].

2.10 Weighing of Animals

The weight of the animals were taken throughout the pre and post ingestion period using the method of Momoh *et al.* [13].

2.11 Haematological Assay

The blood of the infected and uninfected rats was collected weekly into EDTA bottles after which the Packed Cell Volume (PCV), Haemoglobin (HB), Red blood Cell (RBC), White Blood Cell (WBC) and differential leukocytes counts of the collected blood samples were evaluated according to the method described by Baker *et al.* [14]**.**

2.12 Statistical Analysis

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

3. RESULTS AND DISCUSSION

All the rats infected with *S. typhimurium* and treated with honey recovered by the 3rd day while those ones that was administered honey – ORS and the ones with homemade ORS recovered by day 4 and those treated with augmentin and those administered commercial ORS recovered by the 5th day, those that were infected but not treated started to show signs of recovery by the 6th day. The recovery without treatment by the 6th day confirms that acute diarrhoea is self limiting according Chen *et al.* [15]. The mean recovery times of rats infected with *Salmonella typhimurium* and treated with honey –ORS was significantly reduced when

compared with infected and not treated group (Table 1). This is agreement with the work of Beretta *et al.* [16]. There was no evidence of *S. typhimurium* in the faeces of rats treated with 1ml, 2ml, 3ml of honey and administered 1ml of honey –ORS 12hourly for 7 days. **(**Fig.1**).**

Table 1: **Physical observations of the Wistar rats during infection with *Salmonella typhimurium* 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 1ml	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	RA, EL, UF, PM, SS	A, EW, FS, *PM, SS	A, EW, FS, NM, SF	A, EW, FS, NM, SF	A, EW, FS, NM, SF

	homemade (12hourly)							
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

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

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

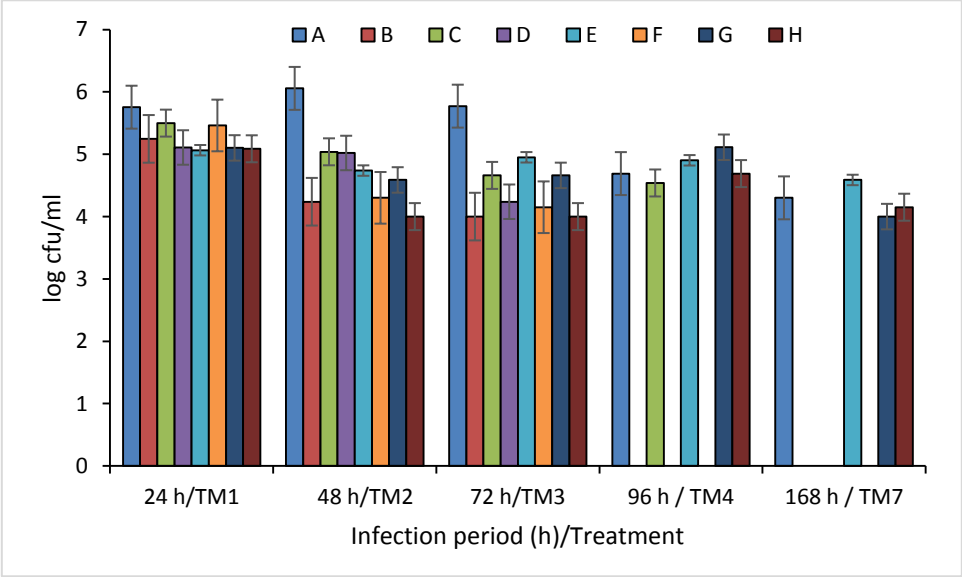
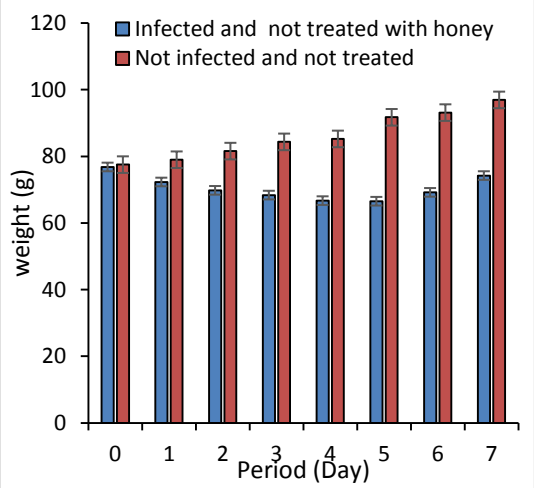


Fig. 1: Total counts of *S. typhimurium* in the faeces of Wistar faeces after infection and treatment

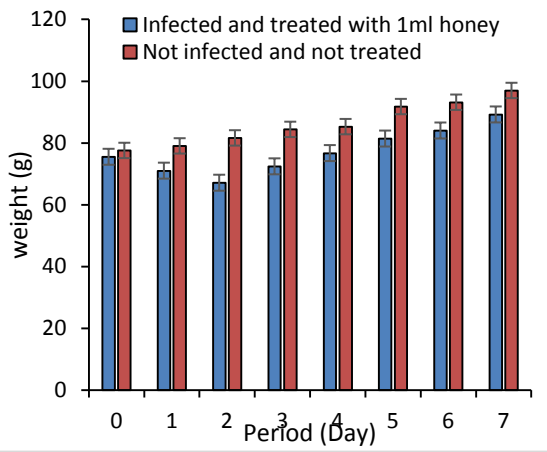
KEY: A = Infected and not treated, B = Infected and treated with 1ml honey, C = Infected and treated with 2ml honey, D = Infected and treated with 3ml honey, E = Infected and treated with 0.5ml augmentin, F = Infected and administered with 1ml honey – ORS, G = Infected and administered with 1ml commercial ORS, H = Infected and administered with 1ml homemade ORS, TM1 = Treatment day 1, TM2 = Treatment day 2. TM3 = Treatment day 3, TM4 = Treatment day 4 and TM7 = Treatment after day 7.

The infected rats lost weight as a result of the infection with *S. typhimurium* however, administration of honey to the infected rats caused a significant increase ($p<0.05$) in their weight but the infected and not treated rats recorded weight loss for a longer duration than those that were administered different volumes of honey (Fig. 2A-E). The observation that the infected and not treated rats did not gain back their body weight throughout the duration of the research is in agreement with the work of Momoh *et al.* [13].

A

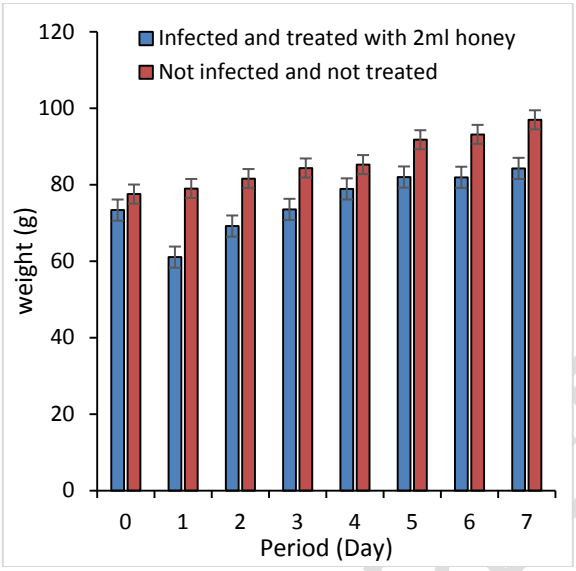


B



128
129
130
131
132
133
134
135
136
137

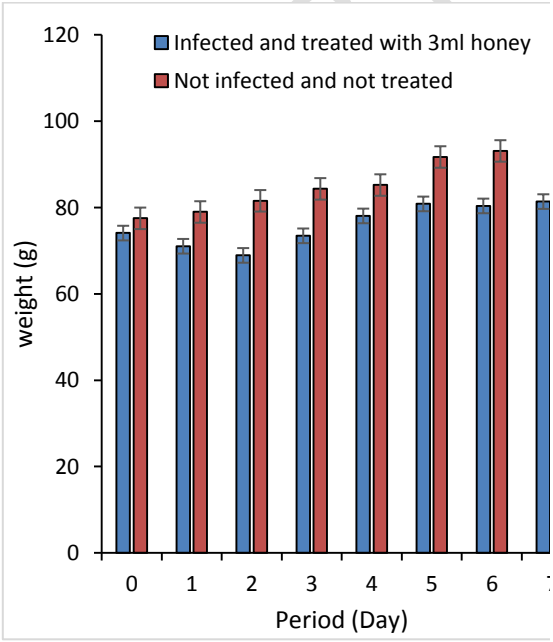
C



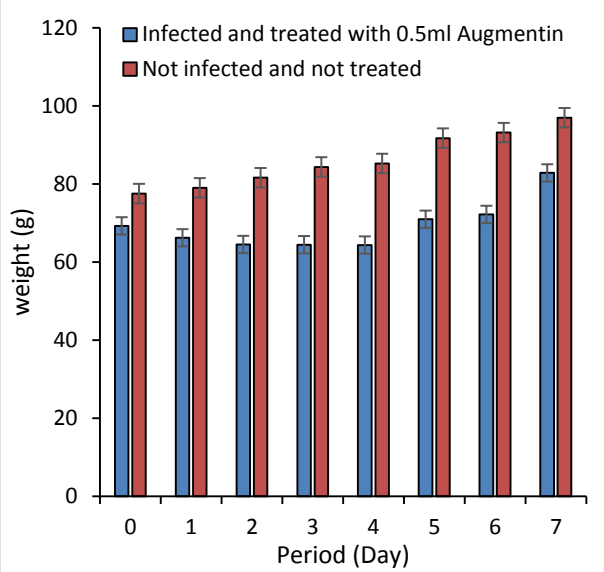
138
139
140

D

142



141



E

Fig, 2 A –E : Average weight of Wistar rats before infection with *S. typhimurium,* at the onset of infection and after treatment with honey and augmentin.

Infection of rats with *S. typhimurium* caused a decrease in their PCV, HB and RBC and increase in their neutrophil counts, showing a sign of infection but after treatment with honey (between 2ml and 3ml), there was no significant difference in the PCV, WBC of the group of rats treated with honey and the group not infected, not treated (control) (Tables 2a and b). Administration of honey to apparently healthy rats (control) caused a significant ($p<.05$) increase in the PCV and lymphocytes of the rats. This shows that the honey has both haematinic and immunomodulatory potentials.

Table 2a: Effect of honey on the haematological parameters of Wistar rats infected with *S. typhimurium*

Group	PCV (%)	HB (g/L)	WBC (10^9/L)	RBC (10^{12}g/L
1	32.50 ± 4.95^{b}	10.85 ± 1.66^{b}	12.70 ± 0.28^{a}	3.00 ± 0.82^{b}
2	40.00 ± 2.83^{ab}	13.50 ± 0.71^{ab}	11.70 ± 0.28^{ab}	3.20 ± 0.25^{ab}
3	42.50 ± 2.12^{a}	14.14 ± 0.66^{a}	8.77 ± 0.49^{c}	4.04 ± 0.87^{ab}
4	43.00 ± 4.24^{a}	14.30 ± 1.36^{a}	8.94 ± 0.42^{c}	4.29 ± 0.07^{a}
5	40.33 ± 4.51^{ab}	13.50 ± 1.51^{ab}	10.86 ± 0.17^{abc}	4.06 ± 0.30^{ab}
6	35.50 ± 0.71^{ab}	11.90 ± 0.28^{ab}	10.88 ± 0.05^{abc}	3.66 ± 0.03^{ab}
7	39.67 ± 4.51^{ab}	13.19 ± 1.56^{ab}	9.56 ± 1.92^{bc}	4.07 ± 0.34^{ab}

8	35.00 ± 6.00^{ab}	11.64 ± 2.00 ^{ab}	9.83 ± 1.47^{bc}	3.66 ± 0.53 ^{ab}
9	42.33 ± 2.08 ^{a}	13.66 ± 0.35 ^{ab}	8.82 ± 1.06^{c}	4.07 ± 0.58 ^{ab}

Key: 1 = Infected and not treated, 2 = Infected and treated with 1ml honey, 3 = Infected and treated with 2ml honey, 4 = Infected and treated with 3ml honey, 5 = Infected and treated with 0.5ml augmentin, 6 = Infected and administered with 1ml honey – ORS, 7 = Infected and administered with 1ml commercial ORS, 8 = Infected and administered with 1ml homemade ORS, 9 = Not Infected, not treated, PCV = Packed cell volume, HB = Haemoglobin concentration, WBC = White blood cell and RBC = Red blood cell.

Table 3b: Effect of honey on the haematological parameters of Wistar rats infected with *S. typhimurium* contd

Group	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	31.50 ± 0.71^{f}	64.50 ± 0.71^{a}	3.00 ± 0.00^{a}	1.50 ± 0.71^{a}	1.00 ± 0.00^{a}
2	37.50 ± 0.71^{bc}	61.00 ± 1.41^{ab}	2.50 ± 0.71^{a}	2.00 ±1.41^{a}	1.00 ± 0.00^{a}
3	40.00 ± 2.83^{ab}	56.50 ± 0.71^{b}	1.50 ± 0.71^{a}	1.00 ± 0.00^{a}	0.00 ± 0.00
4	40.50 ± 0.71^{a}	57.00 ± 1.41^{b}	1.50 ± 0.71^{a}	1.00 ± 0.00^{a}	0.00 ± 0.00
5	39.33 ± 150^{abc}	56.00 ± 4.00^{b}	1.67 ± 1.15^{a}	1.00 ± 0.00^{a}	0.00 ± 0.00
6	37.00 ± 1.41^{cd}	60.00 ± 2.83^{ab}	2.00 ± 1.41^{a}	1.50 ± 0.71^{a}	0.00 ± 0.00
7	32.67 ± 1.15^{ef}	60.33 ± 3.79^{ab}	2.00 ± 1.00^{a}	1.67 ± 0.58^{a}	0.00 ± 0.00
8	34.67 ± 0.58^{de}	60.67 ± 1.15^{ab}	2.33 ± 0.58^{a}	1.67 ± 0.58^{a}	1.00 ± 0.00^{a}
9	40.00 ± 1.00^{ab}	57.33 ± 1.15^{b}	1.33 ± 0.58^{a}	1.33 ± 0.58^{a}	0.00 ± 0.00

Key: 1 = Infected and not treated, 2 = Infected and treated with 1ml honey, 3 = Infected and treated with 2ml honey, 4 = Infected and treated with 3ml honey, 5 = Infected and treated with 0.5ml augmentin, 6 = Infected and administered with 1ml honey – ORS, 7 = Infected and administered with 1ml commercial ORS, 8 = Infected and administered with 1ml homemade ORS and 9 = Not Infected, not treated.

4. CONCLUSION

This study has shown that honey sample from FUNAAB (HF) caused enhanced immune response in the rats against *S. typhimurium*. This honey also has haematinic, immunomodulatory, and immunostimulatory potentials in rats infected with *S. typhimurium.* These findings therefore could be exploited in boosting the immune system and in the treatment of diarrhoeal diseases caused by this bacterium.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

It is not applicable

ETHICAL APPROVAL

As per international standard written ethical approval has been collected and preserved by the author(s).

REFERENCES

1. Torpdahl M, Tsai-Ling L, Shiu-Yun L, Ishien L, Sung-Hsi W, and Chien-Shun C. "Human Isolates of *Salmonella enterica* Serovar *Typhimurium* from Taiwan displayed significantly higher levels of Antimicrobial Resistance than Those from Denmark." International Journal of Food Microbiology. 2013; 69-75.

2. Black JG. Microbiology*:* Principles and Explorations. 8th Edition. 2005. ISBN-13:978-1118285954, ISBN-10:1118285956.

3. Chen C, Campbell LT, Blair SE. and Carter DC.. The effect of standard heat and filtration processing procedures on antimicrobial activity and hydrogen peroxide levels in honey. Frontier's Microbiology. 2012; 3, 265, 1-8.

4. Cheesbrough M. Medical Laboratory Manual for Tropical Countries. VOL. II. Microbiology. 2006; 479. University of Cambridge press. Great Britain.

5. Basualdo, C., Sgroy, V., Finola, M.S., and Junm, M. Comparison of the antibacterial activities of honey from different provenance against bacterial usually isolated from skin wounds. Veterinary Microbiology. 2007; 127: 375-381.

6. Lusby PE, Coombes AL and Wilkinson JM. Bbactericidal activity of different honeys against pathogenic bacteria. Archieve Medical Resources. 2005; 36:464-467.

7. Adebolu TT. Effects of natural honey on local isolates of diarrhoea causing bacteria in Southwestern, Nigeria. African Journal of Biotechnology. 2005; 4(10):1172 - 1174.

8. Boateng J and Diunase KN. Comparing the antibacterial and functional properties of Cameroonian and manuka honey for potential wound healing. Molecules, MDPI Journals 2015; (20)9, 16068 - 16084.

9. Rozanska Hanna. Microbiological Quality of Polish Honey. National Veterinary Research Institute. Pulway. 2011; 55, 443 – 445.

10. Adebolu TT, Adeoye OO, Oyetayo VO. Effect of garlic (*ALLIUM SATIVUM*) ON *Salmonella typhi* infection, gastrointestinal flora and haematological parameters of albino rats. African Journal of Biotechnology. 2011; *1*0 (35):6804-6808.

11. Oladunmoye MK. (2007). The Imunostimulatory effect of ethanolic extract of C*assia alata* on immune system of albino rats dosed with *Staphylococcus aureus* (ncib 8588). Journal of Pharmacology and Toxicology. 2007; 2(2): 200-204.

12. Fawole MO, and Oso BA. Characterization of Bacteria: Laboratory Manual of Microbiology. 4th EDN., Spectrum Book Ltd, Ibadan Nigeria 2004; 24-33.

13. Momoh AO, Adebolu, TT and Ogundare AO. Therapeutic effects of beniseed extracts and fermented liquor in treating diarrhoea in albino rats infected with *Bacillus cereus.* Global advanced research journal of medicinal plants. 2012; 1 (1): 007- 012.

14. Baker FJ and Silverstone RE. Medical Laboratory Science. 8TH EDITION. Chris Publisher, Washington D.C., USA. 2006; 447.

15. Chen, C., Campbell, L.T., Blair, S.E. and Carter, D.C. (2012). The effect of standard heat and filtration processing procedures on antimicrobial activity and hydrogen peroxide levels in honey. Frontier Microbiology. 3, 265, 1-8.

16. Beretta G, Gelmini F, Lodi V, Piazzalunga A, Facino RM. Profile of nitric oxide (no) metabolites (nitrate, nitrite and n-nitroso groups) in Honeys of different botanical origins: nitrate accumulation as index of origin, quality

and of therapeutic opportunities. Journal of Pharmaceutical and Biomedical Analysis. 2010; 53 (3): 343-349.