ASSESSMENT ON IN VIVO ANTI-TYPHOIDAL POTENTIALS OF AQUEOUS EXTRACTS OF LEAVES OF SOME MEDICINAL PLANTS USED IN THE TREATMENT OF TYPHOID FEVER IN ADAMAWA STATE .

Original Research Article

ISSN : 2456-1045 (Online)
(ICV- PHCLS /Impact Value): 63.78
(GIF) Impact Factor: 4.126
Publishing Copyright @ International Journal Foundation
Journal Code: ARJMD/PHCLS/V-22.0/I-1/C-2/FEB-2018
Category : PHARMACEUTICAL SCIENCE
Volume : 22.0 / **Chapter**- II / **Issue** -1 (FEB-2018)
Journal Website: www.journalresearchijf.com
Paper Received: 27.02.2018
Paper Accepted: 07.03.2018
Date of Publication: 10-03-2018
Page: 10-14

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Citation of the Article

Awi M; Tula M.Y; Thomas T.L. (2018) *Assessment on invivo anti-typhoidal potentials of aqueous extracts of leaves of some medicinal plants used in the treatment of typhoid fever in Adamawa state.; Advance Research Journal of Multidisciplinary Discoveries.22.0,C-2 (2018) 10-14*

ABSTRACT

The anti-tyhoidal efficacies of seven (7) medicinal plants from Adamawa State were assessed in vivo in albino rats. Seventy eight (78) albino rats divided into 8 groups (A-H) of 9 rats each and two control group (I and J) of 3 rats each were used. After infection with the typhoid bacilli and confirmed by Widal test, the rats were treated with varying concentrations of each plant extract for 5 days (for plant treated group) and their serum tested again for *Salmonella* agglutinin using Widal test. One control group (group I) were treated with ciprofloxacin and the other (group J) were not treated. The results showed infection with significant titre that varies in all the groups. All the rats in group E, G and H treated with different concentrations of leave extracts of *Acacia* sp., *Carica papaya* and mixture of all the seven plants were completely cured of typhoid as shown by their titres. The effectiveness of other plants species was high and variable. Those that were not cured had their titre reduced significantly. Two (2) out of three rats in Group I treated with ciprofloxacin were also cured of typhoid. All the rats in Group J (not treated) were not cured; one had the titre reduced from 1: 160 to 1:80, but no death was recorded. The mean weight of rats from each group after treatment varies slightly from those before treatment but with no statistical difference (P=0.674).The anti-typhoidal potential was demonstrated in all the plants extract irrespective of their concentrations.

Keywords: anti- typhoidal, medicinal plants, in vivo, typhoid fever.

I. INTRODUCTION

Typhoid fever is a severe life-threatening disease caused primarily by *Salmonella enterica* subspecies enterica serovars Typhi and Paratyphi A, B, or C. The disease is transmitted through faecal-oral route via contaminated water and food, especially by food-handling carriers. Early stage of the disease is characterized by high fever, abdominal pain, loss of appetite, weakness, malaise, dull continuous headache and diarrhea. At advanced stages there is often a protracted fever and mental dullness, peritonitis, intestinal bleeding, slight deafness and parotitis paratyphoid [1]. It is often encountered in tropical countries including Nigeria where they constitute serious sources of morbidities and mortalities [2]. Lack of access to safe water and inadequate sanitation may be important risk factors for typhoid fever.

Treatment of *Salmonella* infection has become challenging because of emerging antibiotic resistance to first-line antibiotics and, more recently, the fluoroquinolones [3]. The emergence of multidrug resistant (MDR) *Salmonella* strains with resistance to fluoroquinolones and third-generation cephalosporins is now a subject of international concern and constitute serious problem that results in severe limitation of the possibilities for effective treatment of human infections. The problem has become endemic in many developing countries, causing enormous morbidity and high cost of treatment [4]. This has resulted in a pressing need to search for alternative and newer antimicrobials with novel mode of action against typhoid fever particularly from plants and their derivatives. Thus, the resistances of *Salmonella* to the outlined antibiotics couple with the high cost of treatment have prompted the present study on plants used for the treatment of typhoid fever in Adamawa State.

II. MATERIALS AND METHODS

Study Area

Mubi is the head quarter of Mubi North Local Government Area of Adamawa State. It is situated in the North Eastern part of Nigeria, between latitude 10° 05' and 10° 30'N of the equator and between longitude 13° 12' and 13° 19'E of the Greenwich meridian. The climate is tropical with average climate of about 32-35^{o}C in dry season and relative humidity ranging from 28-45%mm and an average rainfall of about 1056mm [5], which usually start around May and ends in October. The majority of people of Mubi area are subsistence farmers, cattle rearers and livestock farmers; with few civil servants and business men and women.

Mubi North and Mubi South has an international boundary, east, with the Republic of Cameroon and surrounded by three Local Government Areas of Adamawa State; Maiha, Michika and Hong Local Government Area to the south, north and west respectively.

Collection of Plant Materials

The leaves of all the plants (*Mangifera indica*, *Psidium guajava*, *Carica papaya*, *Acacia* sp, *Musa paradisiaca, Moringa oleifera* and *Citrus sinensis*) were collected at the end of the rainy season (4th - 8th October, 2017) around Gipalma area of Mubi metropolis. All the plants parts were identified and authenticated in the Botany unit of Department of Biological Science Technology, Federal Polytechnic Mubi, Adamawa State., Nigeria.

Extraction Procedure

The leaves of all the plants were properly washed, air-dried at room temperature and ground to fine powder. 50 g of each of the powdered plant part was soaked in 200 ml distilled water and was allowed to stand for 48 hrs at room temperature after thorough vortexing. Each mixture was filtered using whatman no.1 filter paper. The filterate were concentrated in vacuo using rotary evaporator. The aqueous dried extracts of all the plants part were properly labelled and stored in sample bottles at 4°C prior to use

Animal study

Seventy eight (78) albino rats of both sexes weighing between 80 – 207g were employed for this study. They were obtained from the Department of Biochemistry, Modibbo Adama University of Technology (MAUTECH), Yola. All the rats were kept in the Biology Laboratory of Federal Polytechnic Mubi to acclimatize for two weeks before the commencement of the study. The research was also approved by the Institution's Research Committee. Animal care and handling were in compliance with the international guidelines [6].

In-vivo Anti-typhoidal Assay of Crude Extracts of the medicinal plants

Salmonella Typhi obtained from Mubi general hospital was maintained by plating in Deoxycholate citrate agar (DCA) slant and replicated on nutrient agar slant.

Seventy eight (78) albino rats divided into 8 groups (A-H) of 9 rats each and two control group (I and J) of 3 rats each were used. Each group (aside the control groups) was distributed into 3 treatments with 3 rats in each treatment. The treatments for each group were designated with subscript 1, 2 and 3. Each treatment in a group was kept in a separate apartment with free and daily access to food and water *ad libitum*. Also, 6 rats distributed into two groups of 3 rats each were used for the control groups designated I and J. The rats in each group were injected intravenously with the typhoid bacilli (*Salmonella* Typhi). Widal test was carried out on all the groups to ascertain the level of infection with the typhoid bacilli. After infection, all the groups were placed on oral therapy (except group J) daily for a period of 5 days with different concentrations of the plants extracts for plants treated groups (A-H) and Ciprofloxacin for group I. Group A. B, C and D were treated with *Psidium guajava*, *Citrus sinensis*, *Mangifera indica* and *Moringa* oleifera leaves extracts respectively. Similarly, *Acacia* sp, *Musa paradisiaca, Carica papaya* and mixture of all the 7 plants leaf extracts were used to treat group E, F, G and H respectively. Treatment 1, 2 and 3 of each group (A-H) were treated with 100mg/kg, 200mg/kg and 400mg/kg concentrations respectively of the plant extracts of each group. Group I were treated with ciprofloxacin (I_1 with 10, I_2 with 20 and I_3 with 40mg/kg) while Group J were not treated. After oral therapy for 5 days, widal test was carried out again on all the groups to confirm if they were cured of typhoid fever or not. Significant titre for *Salmonella* agglutinin was taken at ≥ 1:80.

Body weight determination

The body weight of all the albino rats in their respective groups was determined after they were infected with typhoid baciili (*Salmonella* Typhi) and also after treatment with the various medicinal plants to assessed weight gain and or weight loss.

Widal test

2 drops of serum to be tested is place on a white tile, the antigen suspension was shaken and 1 drop of the antigen was added. It was then mix over an area of 3cm, rock gently and examine for agglutination after 1 minute. Significant titre was taken for titre ≥1:80.

III. STATISTICAL ANALYSIS

Non-parametric Mann-Whitney statistics was used to determine the significance difference in the weight of the albino rats before and after they were treated. The data were analysed using SPSS version 16.0. Statistical difference was taken when p≤0.05.

IV. RESULT

The result in Table 1 showed the efficacy of all the plants extracts on albino rats as depicted by Widal test result. After infection with the typhoid bacilli, the level of infection with significant titres varies. Sixteen (22.2%), 44.4% and 33.3% of the rats had significant titre of 1:80, 1:160 and 1:320 respectively. After treatment with the plants extracts, 66(91.7%) of the rats were cured completely of typhoid as there was no reaction with their serum. Only 5(6.9%) and 1(1.4%) of the rats had significant titre of 1:80 and 1:160 respectively. The result further showed that all the rats in group E, G and H treated with different concentrations of leave extracts of *Acacia* sp., *C. papaya* and mixture of all the seven plants were completely cured of typhoid as shown by their titres. Similarly, 7(77.8%.7%) rats in group A treated with *P. guajava* leave extract were cured of typhoid, 8(88.9%) rats in group B, C, D and F each treated with leave extracts of *C. sinensis*, *M. indica*, *M. oleifera* and *M. paradisiaca* were also cured of typhoid induced in them. Those that were not cured, had their titre reduced significantly from either 1:320-1:160 (as in the case of group D_2), 1:320-1:80 (as in the case of group A_3 and B_3) or 1:160-1:80 (as in the case of A_2, C_3 and F_3). Two (2) out of three rats in Group I treated with ciprofloxacin were also cured of typhoid. However, the rats treated with 10mg/body weight of ciprofloxacin was not cured completely; rather the titre was reduced from 1:160 to 1:80. All the rats in Group J (not treated) were not cured, one had the titre reduced from 1: 160 to 1:80, they were observed to be weak with raised fur, but no death was recorded.

Table 1: Result of Widal test before and after treatment for plants extracts

Group	No of Albino rats/group	BEFORE TREATMENT No of rats with *Salmonella* agglutinin			AFTER TREAMENT No of rats with *Salmonella* aggluti nin			No. cured
		1:80	1:160	1:320	1:80	1:160	1:320	
A_1	3	1	1	1	0	0	0	3
A_2	3	1	2	0	1	0	0	2
A_3	3	1	1	1	1	0	0	2
B_1	3	1	2	0	0	0	0	3
B_2	3	1	2	0	0	0	0	3
B_3	3	0	0	3	1	0	0	2
C_1	3	0	1	2	0	0	0	3
C_2	3	1	2	0	0	0	0	3
C_3	3	1	2	0	1	0	0	2
D_1	3	1	1	1	0	0	0	3
D_2	3	1	0	2	0	1	0	2
D_3	3	1	1	1	0	0	0	3
E_1	3	0	1	2	0	0	0	3
E_2	3	1	1	1	0	0	0	3
E_3	3	0	1	2	0	0	0	3
F_1	3	0	3	0	0	0	0	3
F_2	3	1	1	1	0	0	0	3
F_3	3	1	2	0	1	0	0	2
G_1	3	1	1	1	0	0	0	3
G_2	3	0	1	2	0	0	0	3
G_3	3	0	1	2	0	0	0	3
H_1	3	1	1	1	0	0	0	3
H_2	3	1	2	0	0	0	0	3
H_3	3	0	2	1	0	0	0	3
Total	72	16(22.2%)	32(44.4%)	24(33.3%)	5(6.9%)	1(1.4%)	0	66(91.7%)

Legend: all subscript 1= treated with 100mg/kg, subscript 2= treated with 200mg/kg, subscript 3= treated with 400mg/kg, A= treated with *P. guajava*, B= treated with *C. sinensis*, C= treated with *M. indica*, D= treated with *M. oleifera*, E= treated with *Acacia* sp., F= treated with *M. paradisiaca*, G= *C. papaya*, H= treated with mixture of the 7 plants.

Table 2: **Result of Widal test before and after treatment for Control Group**

Group	No of Albino rats/group	BEFORE TREATMENT No of Rats with Salmonella agglutinin			AFTER TREAMENT No of Rats with Salmonella aggluti nin			No. cured
		1:80	1:160	1:320	1:80	1:160	1:320	
I_1	1	0	1	0	1	0	0	0
I_2	1	0	1	0	0	0	0	1
I_3	1	0	1	0	0	0	0	1
J_1	1	0	1	0	0	1	0	0
J_2	1	0	1	0	0	1	0	0
J_3	1	0	1	0	1	0	0	0

Legend: I_1 = treated with Ciprofloxacin (10mg/body weight), I_2 = treated with Ciprofloxacin (20mg/body weight), I_3= treated with Ciprofloxacin (10mg/body weight), $J_{1, 2, 3}$ = not treated.

The result in Table 3 showed the mean weight of rats in each group before and after treatment. The mean weight of each group after treatment varies slightly from those before treatment but with no statistical difference (P=0.674).

Table 3: Body weight of albino rats before and after treatment

Group	Before treatment Mean±MSE (g)[a]	After treatment Mean±MSE (g)[a]
A	177.5±11.79	174.7±9.43
B	115.6±6.49	127.1±7.05
C	118.8±5.53	118.3±5.71
D	199.4±8.83	204.9±18.23
E	155.7±11.97	153.9±7.75
F	141.0±16.58	145.5±13.99
G	121.4±14.48	112.9±4.72
H	121.7±3.26	114.2±3.15
I	110.5±3.49	116.8±3.86
J	121.3±8.34	127.0±7.31

Legend: MSE= mean standard error, g= gramme, a=not significantly different (P=0.674)

V. DISCUSSION

Result from previous studies on these medicinal plants in the same study area showed that these plants possessed various phytochemicals such as tannins, polyphenols, phlobatannins, cardiac glycosides, alkaloids, saponins and flavonoids [7]. Phytochemicals can have complementary and overlapping mechanisms of action in the body including modulation of hormone metabolism, modulation of detoxification enzymes, antioxidant effects and stimulation of the immune system. Several studies have reported that phytochemicals possess antibacterial activity [8, 9, 10] which may have been responsible for the in vivo anti-typhoidal activity portrayed by the plants extracts under study.

Recently, many first line antibiotics use for treatment of typhoid have lost their potency due to the emergence of multi-drug resistant *Salmonella* Typhi which occurred primarily through acquisition and expression of resistance genes. In addition to this, antibiotics are sometimes associated with side effects such as allergic reactions, hypersensitivity and immune-suppression. Therefore, the need to explore plants derivatives for the development of alternative antimicrobial drugs for the treatment of typhoid related illnesses is indispensable.

In this study, the group of experimental animals (Albino rats) treated with aqueous extracts of *Acacia* sp, *Carica papaya* and mixture of the seven (7) plants showed total clearance and 100% protection from *Salmonella* Typhi. This finding collaborates with previous report [11] on *Acacia nilotica* whose findings showed complete clearance of typhoid. A similar studies conducted by [12] showed that aqueous extract of fruits of *Citrus sinensis* confer anti-typhoid activity against *Salmonella* Typhi. In our present study however, leave extract of *C. sinensis* was used instead and the result showed that 8 out of 9 *Salmonella* infected rats were cured completely of typhoid. The marked clearance or reduction of *Salmonella* agglutinin portrayed by other plants extracts (such as *Moringa oleifera*, *Carica papaya*, *Mangifera indica*, *Musa paradisiaca* and *Psidium guajava*) on *Salmonella* infected rats confirmed the anti-typhoidal potency of the plants parts. These findings perhaps add to other plants with anti-typhoidal activity demonstrated in albino rats that includes *Vivex doniana* [13], *Momordica* charantia [14], *Albazia ferruginea* [15], Beniseed [16] among others.

Furthermore, clinical signs of infection were rarely observed in the extract treated rats after the period of treatment. This observation was quite similar to previous reports [13, 16] on the use of some medicinal plants on *Salmonella* infected albino rats. In our study, none of the untreated rats died during the period of treatment but were observed to be weak and feed poorly. This observation contradicts previous findings which revealed that all untreated rats were killed after a period of 5-7days of treatment [11, 13].

The marked clearance and reduction of *Salmonella* agglutinin in the serum of typhoid infected rats treated with various concentrations of plants extracts under study confirmed the antimicrobial potency of the plant and therefore suggests its efficacy in the treatment of typhoid fever.

The observed lack of significant changes on the weight of the experimental animals before and after treatment collaborates with previous findings on *Annona muricata* [17] and *Coriandrum sativum* [18]. This might be due to the fact that the measurement was taken immediately after the last day of the treatment; at this point the rats are still recuperating. They require more time to adjust, regain their appetite, feed well, regain their weight and increase in metabolic activities which will ultimately lead to significant increase in body weight. However, this result is in contrast to previous report which found out that all groups of experimental rats treated with leaf extract of *Momordica charantia* recorded steady increase in body weight [14]. Another report showed that weight gain or loss is a function of the dose administered to experimental animals. They observed an increased in the average body weights of the rats treated with 400 mg/kg and a corresponding decrease in weights of those treated with 200mg/kg of the extract of *Ocimum gratissimum* [19]. Increase in the body weight of infected but untreated rats as seen in our study was unexpected. However, this observation collaborates with previous findings which reported a sudden increase in body weight of infected but untreated rats from the six day [17]. According to them the reason might be due to the recovery of the rats as a result of the fighting activity of the increased White Blood Cell differentials. This observation might be true because *Salmonella* agglutinin titre of one out of the 3 rats in Group J (infected but untreated) drops from 1:160 to 1:80 as shown in our study. This observation can also be liken to that of a human case in which some victims of *E. coli* O157:H7 infection recovered without treatment within 5-10 days [20]. Body weight change serves as a sensitive marker of the general health status of animals and it will be significant if the body weight loss that occurred is more than 10% from the initial weight [17]. Consequently, the non statistical difference observed in body weight of rats given plants extracts in this study indicates that the plants extracts under study does not interfere with the metabolism of the rats.

VI. CONCLUSION

The findings of this study provide pharmacological basis for the use of the plants under study in traditional medicine. The *in-vivo* anti-typhoidal potentials demonstrated by the crude extracts of all the plants and combination of the plants elucidate that the plants under study could serve as an excellent candidate for the development of new drugs for the treatment of diseases associated with *Salmonella* Typhi. We strongly recommend further investigation to determine toxicity level of these plants and to identify the bioactive components responsible for these impressive anti-typhoidal activities together with their mechanism of action.

VII. ACKNOWLEDGEMENT

We wish to thank Chief J.P. Uko, Mr. Husseini Bello, Ayuba John, Elisha Richard, Dennis, Pharm. Marouf whose assistance in this work is commendable. We also thank the Department of Biological Science Technology for breeding mall and other assistance. Most importantly, authors are highly grateful to TETFUND (2011-2014 merged) for sponsorship.

REFERENCES

[1]. **Ackers ML, Puhr ND, Tauxe RV, Mintz ED.** Laboratory-Based Surveillance of Salmonella Serotype Typhi infections in the United States. Antimicrobial resistance on the Rise. JAMA. 2000; 283: 2668-2673.

[2]. **Ibekwe AC, Okonko IO, Onunkwo AU, Donbraye E, Babalola ET, Onoja BA.** Baseline Salmonella agglutinin titres in apparently healthy freshmen in Aw ka, South Eastern, Nigeria. Sci Res Essay, 2008; 3(9): 225-230.

[3]. **Aggarwal A, Vij AS, Oberoi, A.** A three-year retrospective study on the prevalence, drug susceptibility pattern, and phage types of Salmonella enteric subspecies Typhi and paratyphi in Christian Medical College and Hospital, Ludhiana, Punjab. JIACM. 2007; 8:32-35.

[4]. **Tsobou R, Mapongmetsem PM, Voukeng KI, Van Damme P.** Phytochemical screening and antibacterial activity of medicinal plants used to treat typhoid fever in Bamboutos division, West Cameroon. J Appl Pharma Sci. 2015; 5(6): 34-49.

[5]. **Adebayo AA, Tukur AL.** Adamawa State in maps. Paraclete publishers Yola, 1999; p. 215.

[6]. **EEC. Council Directive 86/609/EEC of 24 November 1986** on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Official J Eur Communities 1986; L358: 1-29.

[7]. **Awi M, Tula MY, Terry LT. (2017).** Preliminary efficacy assessment of some selected indigenous plant species of Adamawa State, Nigeria on Salmonella typhi. J Pharma Res Intl. 2017; 20(4):1-10.

[8]. **Tula MY, Azih AV, Iruolaje FO, Okojie RO, Elimian KO,Toy BD.** Systematic Study on Comparing Phytochemicals and the Antimicrobial Activities from Different Parts of V. amygdalina. Afr J Microbiol Res. 2012; 6(43): 7089-7093.

[9]. **Osman OF, Mansour IS, El-Hakim S.** Honey compound for wound care: a preliminary report. Ann Burns Fire Disaster, 2003; 16(3).

[10]. **Farombi EO.** African Indigenous Plant with Chemotherapeutic Potentials and biotechnological Approach to the production of bioactive prophylacudiestic agents. Afr J Biotechnol. 2003; 2(12):662-671.

[11]. **Sarkiyayi S, Abdulrasheed K.** Preliminary Investigation on Anti Typhoid Properties of Acacia Nilotica Leaf Extract. Global J Med Res. 2013; 13 (5): 10-15.

[12]. **Vivek K, Nandini S, Anitha S.** Anti Typhoid activity of aqueous extract of fruit peel Citrus sinensis. Intl J Pharm Res Dev. 2010; 2(9):49-59.

[13]. **Kuta FA, Onochie I, Garba S, Adedeji AS.** An In-Vitro and In-Vivo antibacterial activity of Vitex doniana crude extracts on Salmonella typhi. ATBU, J Sci Tech Edu. (JOSTE), 2016; 4 (2): 17-27.

[14]. **Adeyi AO, Jinadu AM, Arojojoye OA, Alao OO, Ighodaro OM, Adeyi OE.** In vivo and in vitro antibacterial activities of Momordica charantia on Salmonella typhi and its effect on liver function in typhoid-infected rats. J Pharmacog Phytother. 2013; 5(11): 183-188.

[15]. **Sarkiyayi S, Karago J, Hassan R.** Studies on Anti Typhoid Properties of Aqueous Methanol Leaves Extract of Albizia ferruginea (Musase) Intl J Biochem Res Rev. 2011; 1(1): 24-30.

[16]. **Momoh AO, Adebolu TT, Ogundare AO.** Evaluation Of Beniseed Extract and fermented liquor in treatment of diarrhoea in albino rats infected with Salmonella Typhi. Eur J Biol Med Sci Res. 2013; 1(2): 16-23.

[17]. **Dada EO, Faleye OS.** In-vitro and in-vivo Activities of the Ethanol Pulp Extract of Annona muricata (Linn) Fruit in Albino Rats Infected with Salmonella typhi. J Adv Med Pharma Sci. 2016; 5(4): 1-12.

[18]. **Patel D, Desai S, Devkar R, Ramachandran AV.** Acute and sub-chronic toxicological evaluation of hydromethanolic extracts of Coriandrum sativum L. Seeds. EXCLI J. 2012; 11:566-575.

[19]. **Dada EO, Komolafe OI.** In vivo evaluation of the inhibitory effects of Ocimum gratissimum on Salmonella typhi. Intl J Pharma Biol Res. 2013; 4(4):185-191.

[20]. **Centres for Disease Control and Prevention.** Questions and Answers: Sickness caused by E. coli O157:H7; 2006. Available:https:www.cdc.gov/ncidod/dbmd/disease info/Escherichia coli-g.htm

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