

Full Length Research Paper

Effect of *Phyllanthus amarus* leaf extract on alterations of haematological parameters in *Salmonellae typhi* infested wistar albino rats

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Accepted 20 December, 2013

Haematological indices provide crucial information to assessing the well-being of an organism. In this present study, the antihaematotoxic effect of *Phyllanthus amarus* leaf extract on *Salmonellae typhi*-induced haematotoxicity in rats were investigated. The experimental animals were randomly divided into three study groups. Group 1 received feed and water and was not induced with typhoid (negative control). Groups 2 and 3 received, in addition to feed and water, single dose of stock *S. typhi* at a concentration of 10⁶ cfu/ml. After 7 days, Widal test confirmed typhoid infection and rats in Group 2 were not treated with the leaf extract but rats in Group 3 were treated with 750 mg/kg body weight ethanol extract of *P. amarus* for 10 days at the end of which animals were sacrificed and blood obtained for haematological indices using standard laboratory methods. In Group 2 (positive control), there were significant ($P < 0.05$) decrease in red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), neutrophils and increase in platelet, total white blood cell (WBC) and lymphocytes relative to the non-induced negative control. In Group 3, the rats recorded a significantly ($P < 0.05$) higher mean values in RBC count, PCV, Hb, MCH, MCV, MCHC and lower values in platelets, WBC and lymphocytes when compared to the *S. typhi* induced positive control (Group 2). The results suggest that treatment of *S. typhi* infection with ethanol extract of *P. amarus* reverses and ameliorates the haematotoxic effects induced by *S. typhi* infection in rats.

Key words: *Salmonellae typhi*, *Phyllanthus amarus*, Blood cells, antihaematotoxic, rats.

INTRODUCTION

Typhoid fever (also called enteric fever) is an acute life threatening febrile illness caused by the bacterium *Salmonellae enterica typhi* (Kotton, 2007). It is the second most common cause of fever, second only to malaria, particularly in the tropics (Wilcocks and Manson-Bahr, 1972). An estimated two million cases of typhoid

and two hundred thousand related deaths each year have been reported (Crump et al., 2004). It is contracted through contaminated food and vegetables (Crum, 2003). In developing countries like Nigeria, *S. typhi* infection is endemic/prevalence and account for high rate of morbidity and mortality, particularly due to inefficient

water carriage method of sewage disposal (Crump et al., 2004). One challenge of a developing country is provision/or availability of portable water for her citizens which have a negative impact on their sanitation. Poor sanitary and hygiene have been reported to increase the prevalence of *S. typhi* infection while reduced incidence in developed countries has been attributed to high level of hygiene (Kotton, 2007). Gastroenteritis, the most common disease caused by *S. typhi* infection is characterized by nausea, vomiting and diarrhoea (Parry et al., 2002). This is possible as *S. typhi* escape the macrophage cells and enter the spleen, liver and other organs where it thrives and re-enter the blood (Jones and Falkow, 1996). These tissues/organs are prone to damage by bacterial toxins which are released by bacterial cells to the host organism during the process of metabolism. This tends to disrupt the blood components/cells or blood forming tissues.

Blood is one of the specialized body fluid responsible for the transportation of nutrients, oxygen, hormones and other metabolites to the body's cell and metabolic waste products away from those cells to sites of elimination. It is known to be the most important body fluid that regulates various vital functions of the body. Mammalian circulation of blood transports such specific chemical substances as nutrients, gases, minerals, metabolic products and hormones between different tissues and organs (Baynes and Dominiczak, 2005). Available reports showed that haematological profiles of different species of animals may be influenced adversely by diabetic condition (Edet et al., 2011), phenylhydrazine (Sanni et al., 2005), and aqueous leaf extract of *Ocimum gratissimum* (Obianime et al., 2011).

Phyllanthus amarus is a tropical shrub indigenous to the rainforest of Amazon and other tropical areas of the world (Samraj, 2001). It belongs to the family Euphorbiaceae and classified as a type of *Phyllanthus nururi* (Kassuya et al., 2005). The plant has been valued in many countries for its medicinal properties and curative potentials for a variety of ailments such as asthma/bronchial infection (Lizuka et al., 2006), jaundice and hepatitis B and other viral infections (Huang et al., 2003). It exhibits inhibitory effect on human immune virus (HIV) and reverse transcriptase activity (Notka et al., 2004). Nwanjo et al. (2007) has reported the hypotensive, hypoglycaemic and hypocholesterolemic effect of *P. amarus* extract on hepatocytes of diabetic rats while Nwankpa et al. (2012) has reported the antioxidative effect of the plant extract on *S. typhi* induced oxidative stress in rats. The *in vitro* and antimicrobial activity of the plant extract against *Staphylococcus*, *Micrococcus* and *Pasteurella* spp has been reported (Agrawal et al., 2004).

In rural communities in Nigeria, *S. typhi* infection is endemic and people resort to the use of *P. amarus* for the management of typhoid fever and related cases without recourse to the haematological effects. This study was therefore designed to investigate the effects of *P.*

amarus on haematological profiles in *S. typhi* infested albino rats.

MATERIALS AND METHODS

Plant materials

The fresh leaves of *P. amarus* were harvested from the natural habitat in Owerri, Imo State, Nigeria. They were identified and authenticated by Professor S.C. Okeke of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. A voucher samples are kept in the University herbarium for reference.

Preparation of extract

The fresh leaves of *P. amarus* were washed free of sand and debris. Large quantities were dried under shade at room temperature of 27°C for three weeks. The dried leaves were homogenized with an electric blender to get a coarse powder used for the extraction. 700 g of the powder were macerated in 1.1 L of 80% (v/v) ethanol. The mixture was allowed to stand for 24 h after which it was filtered with a chess cloth. The filtrate was concentrated *in vacuo* at low temperature (37 to 40°C) to 10% of its original volume using a rotary evaporator. The concentrate was placed in a water bath (40°C) to evaporate and the solid residue referred to as extract. Approximate concentration of the extract was made in 100 ml of 10% ethanol for the experiment.

Salmonellae typhi

Stock *S. typhi* was obtained from Federal College of Veterinary and Medical Laboratory Technology of the National Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria. The stock *S. typhi* was sub-cultured into nutrient agar plates, cesteine lactose electrolyte deficient plate (DCA). Plates were incubated at 37°C for 24 h and examined for growth. Stock culture slants were then prepared using McCartney bottles and nutrient agar. The organism from the sub-cultured plate was then aseptically incubated for 18 h.

Animals

Albino wistar rats weighing between 150 to 200 g of both sexes maintained at room temperature in the Animal House of the Faculty of Medicine, Imo State University, Owerri, Nigeria were acclimatized for 12 days to daily handling and were fed *ad-libitum* with normal commercial rat chow (Product of Pfizer Nigeria Ltd) and water.

Induction of typhoid

One (1) ml of *Salmonellae typhi* at a dose of 10^6 cfu/ml was orogastrically administered to the rats to induce typhoid (Kirby-Bauer, 1960).

Experimental design

Twenty-four rats used for this study were randomly assigned into three groups of eight animals each.

Group 1: Rats in this group were not induced with typhoid fever and were fed with normal commercial rat chow and has free access

Table 1. Physical examination/observation of the rats in both experimental and control groups.

Group	Treatment	Soft and mucous feecal matter	Loose and erect hairs	Vomiting
1	Negative control (water)	-	-	-
2	<i>Salmonellae typhi</i> (positive control)	+	+	-
3	<i>Salmonellae typhi</i> + <i>Phyllanthus amarus</i>	-	-	-

+ = Present; - = Absent.

Table 2. Serology test of rats in Groups 2 and 3 infected with *Salmonellae typhi* before treatment with *Phyllanthus amarus* leaf extract.

Group	<i>Salmonella</i> antigen	Antibodies	
		O	H
2	<i>Salmonella Paratyphi A</i>	-	-
	<i>Salmonella Paratyphi B</i>	-	-
	<i>Salmonella Paratyphi C</i>	-	-
	<i>Salmonella typhi</i>	+	+
3	<i>Salmonella Paratyphi A</i>	-	-
	<i>Salmonella Paratyphi B</i>	-	-
	<i>Salmonella Paratyphi C</i>	-	-
	<i>Salmonella typhi</i>	+	+

+ = agglutination (*Salmonella typhi* present), - = No agglutination (*Salmonella typhi* absent); Titre values $\geq 1/160$ were considered positive.

to water throughout the period of the experiment. They were used to monitor successful induction of typhoid.

Group 2: The rats in this group served as control. They were fed with normal rat chows and orogastrically given single dose of *S. typhi* at 10^6 cfu/ml. After 7 days of infection, the rats were observed to have loose and erect hairs as well as soft mucous feecal matter signifying signs of infection and diarrhoea. The rats in this group were not treated with the plant extract.

Group 3: The rats in this group were fed with normal rat chow and orogastrically given single dose of *S. typhi* at 10^6 cfu/ml. After 7 days of infection, the rats showed signs of infection as the rats in Group 2. Serology test, tube agglutination method (Cheesborough, 2005) were used to test for O & H antibodies using a commercial prepared antigen suspension (BSL Global Plasmatic, UK. Code FAT 1010 and 1002 for O & H respectively) to confirm *S. typhi* infection after which they were orogastrically given 750 mg/kg ethanol leaf extract of *P. amarus* daily for 10 days.

Collection and preparation of blood samples for analyses

Twenty-four hours after the last treatment was given, all the rats were weighed and quickly sacrificed under chloroform vapour anaesthesia. With a sterile syringe and needle, 5 mls of blood was collected from each animal by cardiac puncture into EDTA treated screw-cap sample bottles. The anti-coagulated blood samples were used for haematological analyses which were carried out within 24 h of sample collection.

Haematological analysis

Full blood counts such as packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), total white blood cells (TWBC), platelet count, differential white blood cell (lymphocytes, monocytes, neutrophils, eosinophils) and red cell indices including mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean cell haemoglobin concentration (MCHC) were estimated using the Sysmex® Automated Haematology Analyzer KX-2IN, Sysmex Corporation, Kobe, Japan.

Statistical analysis

Statistical evaluation of the data generated was carried out using one-way analysis of variance of the SPSS window Statistical software Programme. This was followed by the student's t-test of significance. Values were declared significantly different at $P < 0.05$.

RESULTS

The results of physical examination of the rats in uninfected and untreated, infected and untreated as well as infected and treated groups are shown in Table 1. The infected and untreated rats (Group 2) were observed to have loose and erect hairs (a sign of fever) and soft and mucous feecal matter (a sign of diarrhoea). These were not observed in uninfected and untreated (Group 1) and infected and treated rats (Group 3). However, the rats in all the groups showed no sign of vomiting. Serology result of the rats in Group 3 is shown in Table 2. The result confirms the presence of O and H antibodies in the serum of the rats indicative of typhoid fever.

Tables 3 and 4 shows the effect of *S. typhi* infection and subsequent treatment with 750 mg/kg body weight daily ethanol leaf extract of *P. amarus* on haematological parameters in albino rats. The results showed a significant ($P < 0.05$) decrease in red blood cells (RBC) count, packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and percentage neutrophil levels in *Salmonellae typhi* infested rats compared to the non-infested group (Tables 3 and 4). On the contrary, the total white blood cell (WBC), platelets and lymphocyte levels in rats infested with *S. typhi* showed a significant ($P < 0.05$) increase compared to the non-infested group (Table 3). Treatment of the rats in Group III with ethanol leaf extract of *P. amarus* showed a

Table 3. Effect of *Phyllanthus amarus* on mean values of red blood cells, packed cell volume, hemoglobin and red cell indices in both experimental and control groups.

Group	Treatment	RBC ($\times 10^{12}/L$)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
1	Negative control (water)	4.02 \pm 0.13	15.6 \pm 1.35	48.33 \pm 1.14	60.13 \pm 1.52	18.54 \pm 1.21	33.27 \pm 1.11
2	<i>Salmonellae typhi</i> (positive control)	1.70 \pm 0.65 ^a	10.53 \pm 1.20 ^a	32.16 \pm 1.12 ^a	49.18 \pm 1.13 ^a	12.78 \pm 1.38 ^a	25.16 \pm 0.89 ^a
3	<i>Salmonellae typhi</i> + <i>Phyllanthus amarus</i>	3.89 \pm 0.21 ^{bc}	14.95 \pm 0.51 ^{bc}	47.13 \pm 0.8 ^{bc}	58.85 \pm 1.28 ^{bc}	17.18 \pm 0.82 ^{bc}	32.16 \pm 1.22 ^{bc}

Mean \pm SD (n = 8); ^a Significantly different compared with negative control (P < 0.05); ^b Significantly different compared with positive control (P < 0.05); ^c No significant difference compared with negative control (P > 0.05).

Table 4. Effect of *Phyllanthus amarus* on mean values of platelets, total white blood cells and differential cell counts in both experimental and control groups.

Group	Treatment	Platelets ($\times 10^3/\mu L^{-1}$)	TWBC ($\times 10^3/\mu L^{-1}$)	Lymphocytes (%)	Neutrophils (%)	Eosinopils (%)	Monocytes (%)
1	Water (Negative control)	850.18 \pm 1.51	14.15 \pm 0.81	73.65 \pm 1.56	22.56 \pm 1.30	2.85 \pm 0.67	2.68 \pm 0.72
2	<i>Salmonellae typhi</i> (positive control)	872.56 \pm 1.61 ^a	23.82 \pm 1.40 ^a	85.72 \pm 1.23 ^a	12.52 \pm 1.12 ^a	3.21 \pm 0.13 ^c	2.90 \pm 0.33 ^c
3	<i>Salmonellae typhi</i> + <i>Phyllanthus amarus</i>	848.17 \pm 1.35 ^{bc}	16.47 \pm 0.6 ^{bc}	72.56 \pm 1.56 ^{bc}	21.68 \pm 0.81 ^{bc}	2.75 \pm 0.15 ^c	2.79 \pm 0.75 ^c

Mean \pm SD (n = 8); ^a Significantly different compared with negative control (P < 0.05); ^b Significantly different compared with positive control (P < 0.05); ^c No significant difference compared with negative control (P > 0.05).

significant (P < 0.05) increase in RBC count, Hb, PCV, MCH, MCV, MCHC and percentage neutrophil levels compared to the *S. typhi* infested non-treated (positive control) group (Tables 3 and 4). However, the treatment of rats in Group III with ethanol leaf extract of *P. amarus* showed a significant (P < 0.05) decrease in platelets, WBC and lymphocyte levels compared to the non-treated *S. typhi* infested group (positive control) Table 3. The results obtained in this study showed no significant (P > 0.05) difference in RBC, Hb, PCV, MCV, MCH, MCHC, platelets, WBC, and lymphocytes in *S. typhi* infested rats treated with the plant extract, compared to the non-infested rats (Tables 3 and 4).

DISCUSSION

Blood is known to be the most important body fluid that regulates various vital functions of the body including transport of metabolic substances and defence against foreign substance, among others. Nutritional, environmental and bacterial infection are among several other factors which have been shown to have adverse effects on the haematological indices of most organism (Jee et al., 2005; Uboh et al., 2009; Savithri et al., 2010). Bacterial infection in living cells cause cellular damage to the host organism by the release of toxins which alter the process of host metabolism and in most cases lead to an increase in free radical species (Stipanuk, 2000). In this study *S. typhi* infection significantly decreases the mean levels in RBC, PCV, Hb, MCV, MCH, MCHC, neutrophils and increase in WBC and lymphocytes which agrees with the symptoms of fever and diarrhoea observed in physical examination of the rats. The observation made

in this study agrees with the report of Wlicocks and Manson-Bahr (1972) on *S. typhi* infection and Kumar and Kuttan (2005) on cyclophosphamide – induced toxicity. The haematotoxic effect of *S. typhi* infection was due to the interaction of the bacterium or its toxins with the blood forming tissues/organs which inhibit the rate at which some specific or generalized haematopoietic committed stem cells are synthesized by the tissues. This was connected to the damage of the tissues, particularly haematopoietic tissues by the bacterium. Benzene and cyclophosphamide-induced haematotoxic effects have been reported to be associated with the interaction of their metabolites with the haematopoietic tissues which cause suppression and depression of their haematopoietic activities (Synder and Hedli, 1996; Kumar and Kuttan, 2005). The reports showed that the metabolites of these chemicals can interact with the red blood cell membrane proteins to increase the rate of red blood cell destruction. Therefore, the decrease in RBC counts, Hb and PCV observed in this study were due to retarded haematopoiesis, destruction and shrinkage of RBC while the decrease in MCV, MCH and MCHC may likely be due to destruction of RBC and decrease in Hb synthesis and haemoglobin content. The observed result is an indication of anaemic condition. Significant increase in total white blood cell and lymphocytes as well as decrease in neutrophils observed in this study is consistent with the reports on the effect of insecticides and pesticides such as fenvalerate, aldrin and lidane on total white blood cells and the differential counts in experimental animals (Synder and Hedli, 1996; Kumar et al., 1996; Savithri et al., 2010). This was explained by increased lymphopoiesis and or enhanced release of lymphocytes from lymph myeloid tissue (Das and

Mukherjee, 2003). This response may be a direct stimulatory effect of toxic substance on lymphoid tissue or chemical (toxin) induced tissue damage and disturbance of the non-specific immune system leading to increase in production of leukocytes. Neutrophils are known to be involved in the phagocytosis of foreign chemical substances in the body during which some of them are ruptured. This explains the observed decrease in neutrophil count on infection with *S. typhi*.

Ethanol leaf extract of *P. amarus* significantly increased the level of RBC, Hb, PCV, MCV, MCH and MCHC thereby reversing/ameliorating the anaemic condition induced by *S. typhi* infection. The rats were observed to recover from fever and diarrhoea. The observed increase in RBC, Hb, and PCV recorded in this study on administration of ethanol leaf extract of *P. amarus* were due to reversal of bone marrow depression thus improving haematopoietic activity of the cells and the improvement in erythrocyte membrane integrity through the antioxidant potential of the extract, thereby reducing haemolysis (Naaz et al., 2007; Nwankpa et al., 2012). Also bacterial infection causes deoxyribonucleic acid disintegration and has been shown to be ameliorated by the bacteriocidal effects of the extract (Okigbo and Ajalie, 2005), leading to an increase in protein synthesis and cell proliferation (Rajinder et al., 2008). Increase in protein synthesis may as well explain the increase in the level of Hb observed in this study. Expectedly, increase in RBC count on administration of *P. amarus* extract results to increase in MCV while increase in Hb results to increase in MCH and MCHC. In this investigation, it was observed that there were significant decrease in total white blood cell, lymphocytes and an increase in neutrophils on administration of *P. amarus* extract on *S. typhi* infected rats. The decrease in WBC and lymphocytes may be due to the inhibition of growth of *S. typhi* (bactericidal effect) by the plant extract leading to the destruction of WBC and lymphocytes. Similar results have been reported on the inhibition of growth of some human pathogens by the plant extract (Notka et al., 2004; Agrawal et al., 2004). However, the increase in neutrophil may be explained by reduced phagocytosis of the microbial cell by neutrophil due to the drastic reduction in microbial growth.

Conclusion

Adverse effects on haematological profiles of an individual may predispose the individual to anaemia. This study has established that ethanol leaf extract of *P. amarus* reverses anaemic condition induced by *S. typhi* infection in albino rats. This lends credence to recovery from fever and diarrhoea.

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