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Effect of *Chromolaena odorata* leaf extract on haematological profiles in *Salmonellae typhi* infested Wistar rats

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ABSTRACT:

Haematological indices provide an insight about the internal environment of a given organism. In this present study, the possible anti-haemototxic effect of *Chromolaena odorata* on *Salmonellae typhi* – induced haematotoxicity in rats were investigated. The experimental animals were divided into three groups. Group A received only food and water (control). Group B and C received in addition to food and water, single dose of stock *Salmonellae typhi* at a dose of 10^6cfu/ml. The animals in group B and C were allowed to be infected with *Salmonellae typhi* for 7 days and confirmed by widal test, after which group C was treated with 750mg/kg body weight/ day ethanolic extract of *Chromolaena odorata* for 10 days. The result 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 haemoglobin Concentration (MCHC), neutrophil and increase in platelet, total White Blood Cell (WBC) and lymphocytes in animals infected with *Salmonellae typhi* when compared to the control non-infected group. Treatment of animals in group C with ethanolic extract of *Chromolaena odorata* showed a significant ($P < 0.05$) increase in mean values of RBC count, PCV, Hb, MCH, MCV, MCHC and decrease in platelets, WBC and lymphocytes when compared to the group infested with *Salmonellae typhi* only. The results above suggest the anti-haematotoxic potential of ethanolic extract of *Chromolaena odorata* in *Salmonellae typhi* infested rats.

Keywords:
Salmonellae typhi, Chromolaena odorata, Blood cells, Anti-haematotoxic, Rats.

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INTRODUCTION

Enteric fever, also called typhoid fever caused by the bacterium *Salmonellae typhi*, is an acute life threatening febrile ailment (Kotton, 2007). Typhoid fever is distributed worldwide and prevalent throughout the tropics where it is the commonest cause of fever (Wilcocks and Manson-Bahr, 1972). Literature reports have shown that two million cases of typhoid and 200 thousand related deaths occur worldwide each year (Steinberg *et al.,* 2004). One challenge of development in developing countries, is the provision of portable water for the populace as poor sanitary condition and hygiene has been reported to increase the prevalence of *Salmonellae typhi* infection with reduced incidence in developed countries (Kotton, 2007). Available reports indicate that typhoid infection is the leading cause of morbidity and mortality in a developing country like Nigeria where water carriage method of sewage disposal is inefficient (Crump *et al.,* 2004). *Salmonellae typhi* infection causes gastroenteritis which symptoms include nausea, vomiting and diarrhea (Parry *et al.,* 2002). The affected organs include spleen, liver and other tissues which habor the bacterium before entering the blood (Jones and Falkow, 1996). During metabolism, bacterial cells, release chemical toxins which interactions damage the tissue of the host organism. This tends to disrupt the blood components 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 such as excretion, respiration, circulation, osmotic and temperature balance etc. Mammalian circulation of blood transports specific nutrients, gases, metabolic products and hormones between different tissues and organs (Baynes and Dominiczak, 2005). Literature reports indicated that haematological profiles of different species of animals may be influenced adversely by diabetic condition (Edet *et al.,* 2011), phenylhydrazine (Sanni *et al.,* 2005), some anti-retroviral drugs (Kayode *et al.,* 2011) and aqueous leaf extract of *Ocimum gratissimum* (Obianime *et al.,* 2011).

Chromolaena odorata (known as siam weed, independent weed, killer weed) is a perennial shrub which grow in rainforest, grassland and arid bushvelds (Timbilla and Braimah, 2002). The leaves of the plant has been reported to be widely used as herbal remedy for the treatment of various ailments. Available reports have shown a decotion of the leaf extract effective in the treatment of malaria and cough (Suksamran *et al.,* 2004). Akah (1990) has reported the haemostatic and anti-inflammatory property of the leaf extract while Thang *et al.,* (2005) has shown the stimulation of granular tissue and re-epithelization of the epithelial tissue during wound healing. Recently Nwankpa *et al.,* (2012) reported the antioxidative effect of ethanolic leaf extract of *Chromolaena odorata* in rats. Other medicinal uses including anti-hypertensive, anti-diarrhoeal and diuretic has been reported (Iwu, 1993).

In rural communities in Nigeria, the use of *Chromolaena odorata* for treating *Salmonellae typhi* infection is common but the effect of the plant on haematological indices in typhoid fever is not known. This study was therefore designed to assess the effect of *Chromolaena odorata* on haematological profiles in *Salmonellae typhi* infested rats.

MATERIALS AND METHODS

Plant Material: The *Chromolaena odorata* leaves were collected from a natural habitat in Owerri and authenticated by professor S.C. Okeke, a taxonomist at the department of Plant Science and Biotechnology, Imo State University Owerri, Nigeria. The voucher specimen was kept in the university herbarium for references.

Preparation of Extract: Large quantities of fresh leaves of *Chromolaena odorata*, washed free of sand and

debris, were dried under shade at room temperature at 27°C for 3 weeks. Electric blender was used to homogenize the dried leaves to a powder form. A 700g of the powder macerated in 1.1 litres of 80% (v/v) ethanol were allowed to stand for 24 hours. A chess clot was used to filter the mixture and the filtrate concentrated in vacuo at 37-40°C to 10% its original volume using a rotary evaporator. The concentrate was evaporated in a water bath at 40°C to a solid residue, the extract. The extract was dissolved in 100ml of 10% ethanol to an approximate concentration used for the experiment.

Salmonellae typhi: The stock *Salmonellae typhi* was procured from Federal College of Veterinary and Medical Laboratory Technology of the National Veterinary Research Institute Vom, Jos, Plateau State, Nigeria. Nutrient agar plate, cesteine lactose electrolyte deficient plate (DCA) was used to sub-culture the micro-organism which was incubated at 37°C for 24 hours and examined for growth. The stock sample used for the experiment was prepared as culture slants using McCartney bottle and nutrient agar. *Salmonellae typhi* from the sub-cultured medium was aseptically incubated for 18 hours at 37°C.

Animals: Albino Wistar rats of both sexes weighing between 150-200g were obtained from the animal house of Faculty of Medicine, Imo State University Owerri, Nigeria. They were maintained at room temperature and acclimatized for 12 days to daily handling. They were fed *ad-libitum* with commercial rat chow (Product of Pfizer Nigeria Ltd) and had free access to water.

Induction of typhoid: Each rat was orally administered with 1ml of *Salmonellae typhi* at a dose of 10^6cfu/ml to induce typhoid (Kirby, 1960).

Experimental design: Twenty - four albino Wistar rats were used for the study. They were randomly assigned into 3 groups. Each group has 8 rats.

Group A: The rats in this group were fed with rat chow and had free access to water. They were not administered with *Salmonellae typhi* and serve to monitor successful induction of typhoid.

Group B: The rats in this group served as control. They were fed with rat chow and had free access to water. Single dose of *Salmonellae typhi* at10^6cfu/ml was orally administered to rats in this group but were not treated with the plant extract.

Group C: The rats in this group were fed with rat chow and had access to water. Single dose of *Salmonellae tysphi* at 10^6cfu/ml were orally administered to the rats in this group. After 7 days of infection, 750 mg/kg ethanolic leaf extract of *Chromolaena odorata* were orally administered to the animals daily for 10 days.

Collection and preparation of blood samples for analysis

At the end of the treatment, the animals were fasted for 24 hours, re-weighed and sacrificed under chloroform anesthesia. By cardiac puncture, blood sample was collected from each animal with a sterile syringe and needle, in EDTA anti coagulated bottle. The anti-coagulated blood samples were used for haematological analyses which were carried out within 24 hours of sample collection.

Haematological analysis

Full blood counts such as packed cell volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), Total White Blood Cells (TWBC), Platelet count, differential white blood cell (like lymphocytes, monocytes, eosinophils, neutrophils) 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

Data generated were statistically analysed by one-way analysis of variance (ANOVA) of the SPSS statistical programme of Microsoft Excel. Values were

declared significantly different at p<0.05.

RESULTS AND DISCUSSION

Table 1 and 2 shows the effect of *Salmonellae typhi* infection and subsequent treatment with ethanolic leaf extract of *Chromolaena odorata* on haematological parameters in rats. The results showed a significant (P < 0.05) decrease in Red Blood Cells (RBC) count, haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and percentage nuetrophil levels in *Salmonellae typhi* infested rats compared to the non-infested group (Table 1 and 2). On the contrary, the total White Blood Cell (WBC), platelets and lymphocyte levels in rats infested with *Salmonellae typhi* showed a significant (P < 0.05) increase compared to the non-infested group (Table 2). Treatment of the rats in group C with ethanolic leaf extract of *Chromolaena odorata* showed a significant (P < 0.05) increase in RBC count, Hb, PCV, MCH, MCV, MCHC and percentage neutrophil levels compared to the *Salmonellae typhi* infested non-treated group (Table 1 and 2) while treatment of rats in group C with ethanolic leaf extract of *Chromolaena odorata* showed a significant (P < 0.05) decrease in platelets, WBC and lymphocyte levels compared to the non-treated *Salmonellae typhi* infested group (Table 2). However the results of this study showed no significant (P > 0.05) difference in RBC, Hb, PCV, MCV, MCH, MCHC, platelets, WBC, and lymphocytes in *Salmonellae typhi* infested rats treated with *Chromolaena odorata* compared to the non-infested rats (Table 1 and 2).

Haematological indices provide relevant information regarding the internal milieu of an organism. Nutritional, environmental and microbial infection are among several other factors which have been reported to have adverse effects on the haematological profiles of most organisms. Vitamin B_{12} and folic acid deficiency (Jee *et al.,* 2005, Murray *et al.,* 2007) and exposure to environmental pollutants such as carbondisulphide, insecticide, hexane, gasoline vapour, nitrocellulose thinner has been reported (Dhembara and Pandhe, 2000; Uboh *et al.,* 2007; 2009; 2012 and Savithri *et al.,* 2010). Bacterial infection in living cells release toxins which metabolism results to increase in release of free radical species with attendant damage to the cells (Stipanuk, 2000). In this study, *Salmonellae typhi* infection significantly decreases the level of RBC, PCV, Hb, MCH, MCV, MCHC, neutrophils and increases the level of WBC and lymphocytes. The observation made in this study agrees with the report of Wilcocks and Manson-Bahr (1972) in *Salmonellae typhi* infection and Kumar and Kuttan (2005) on cyclophosphamide induced

Table 1: Effect of *Chromolaena odorata* on mean values of red blood cells, packed cell volume, hemoglobin and red cell indices in both experimental and control groups.

Group	Treatment	RBC X10^{12}/L	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
A	Negative control/water	3.69 ± 0.21	14.43 ± 0.65	44.33 ± 2.13	63.12 ± 1.60	17.19 ± 1.12	31.27 ± 1.20
B	*Salmonellae typhi* (Positive control)	1.62 ± 0.03[a]	10.09 ± 0.71[a]	33.26 ± 2.14[a]	54.85 ± 1.55[a]	12.52 ± 1.30[a]	24.12 ± 1.23[a]
C	*Salmonellae typhi* + *Chromolaena odorata*	3.49 ± 0.05[bc]	14.15 ± 0.79[bc]	43.40 ± 2.34[bc]	61.95 ± 1.32[bc]	16.55 ± 1.02[bc]	30.12 ± 1.33[bc]

Mean ± SD (n = 8)
[a] Significantly different compared with negative control (P < 0.05).
[b] Significantly different compared with *Salmonellae typhi* (positive control) (P < 0.05).
[c] No significant difference compared with negative control (P > 0.05).

Table 2: Effect of CO on mean values of platelets, total white blood cells and differential cell counts in both experimental and control groups

Group	Treatment	Platelets $X10^3\mu L^{-1}$	TWBC $X10^3\mu L^{-1}$	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Monocytes (%)
A	Negative control/ water	855.18 ± 2.11	16.24 ± 0.78	70.11 ± 2.01	20.19 ± 1.15	1.98 ± 0.6	2.51 ± 0.11
B	*Salmonellae typhi* (Positive control)	880.13 ± 1.5[a]	25.85 ± 1.16[a]	82.14 ± 2.11[a]	11.56 ± 0.87[a]	3.20 ± 1.10	2.90 ± 0.55
C	*Salmonellae typhi* + *Chromolaena odorata*	858.82 ± 1.46[bc]	17.14 ± 1.21[bc]	72.18 ± 1.88[bc]	19.26 ± 1.11[bc]	2.10 ± 0.80	2.6 ± 0.52

Mean ± SD (n = 8)
[a] Significantly different compared with negative control (P < 0.05).
[b] Significantly different compared with *Salmonellae typhi* (positive control) (P < 0.05).
[c] No significant difference compared with negative control (P > 0.05).

toxicity. The haematotoxic effect of *Salmonellae typhi* infection may be explained by the interaction of the bacteria or its toxins with the blood forming tissues/ organs which may inhibit the rate at which some specific or generalized haemopoeitic committed stem cells are synthesized by the tissues. Some reports have shown that hexane, cyclophosphamide and benzene induced haematotoxic effect is associated with the interaction of their metabolites with the haematopoeitic tissues and cause depression in their haematopoeitic activities (Synder and Hedli, 1996; Kumar and Kuttan, 2005). Increase in total white blood cells and lymphocytes as well as decrease in neutrophils seen in this study is consistent with the reports on effect of insecticides and pesticides such as fenvalerate, lindane, aldrin among others, 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 may be explained by increased lymphopoeisis 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/ pollutant induces 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 substances in the body during which some of them are ruptured. This may explain the decrease in neutrophil count on infection with *Salmonellae typhi*.

Ethanolic extract of *Chromolaena odorata* significantly increased the level of RBC, Hb, PCV, MCV, MCH and MCHC thereby reducing and ameliorating the anaemic condition induced by *Salmonellae typhi* infection. The observed increase in RBC, Hb, and PCV may be explained by the role of *Chromolaena odorata* extract in reversing bone marrow depression with attendant improvement in erythrocyte membrane stability through the antioxidant potential of the plant extract, thus reducing haemolysis (Krause and Mahan, 1984; Naaz *et al.,* 2007, Nwankpa *et al.,* 2012). The improvement on the haematopoetic activities of the tissues and/or maintenance of red blood cell membrane integrity relieves the anaemic condition observed in *Salmonellae typhi* infection.

Consequently, increase in RBC count on administration of *Chromolaena odorata* leaf extract translates to an increase in MCV while increase in Hb translates, to an increase in MCH and MCHC. Furthermore, inhibition of microbial growth by the plant extract has been reported. Okigbo and Ajalie (2005) and Alisi *et al.,* (2011) showed that *Chromolaena odorata* leaf extract possess antibacterial activity which inhibit the growth of *Salmonellae typhi* in cells. Decrease in total white blood cell, lymphocytes and attendant increase in neutrophils on administration of the plant extract may be explained by the inhibition of growth of

Salmonellae typhi in the cell. The inhibition of growth of the microorganism lead to the destruction of excess WBC and lymphyocytes released by the cell in response to bacterial infection (Nancy *et al.,* 2005). Conversely, increase in neutrophil count on administration of the plant extract may be explained by reduced phagocytosis of the microbial cell consequent upon drastic reduction in the growth of microbial cell.

CONCLUSION

This study has established the anti-haematotoxic potential of ethanolic leaf extract of *Chromolaena odorata* against *Salmonellae typhi* induced haematotoxicity in rats.

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