THE EFFECTS OF SUB-CHRONIC ADMINISTRATION OF THE ETHANOLIC EXTRACT OF *Chromolaena odorata* LEAVES ON THE IMMUNE SYSTEM AND SPLEEN OF MALE WISTAR RATS

ABSTRACT

Chromolaena odorata, a neglected weed with wide spectrum is a multipurpose plant that contains high concentration of anti-inflammatory, anti-diarrheal, anti-hypertensive agents that protects against tissue damage. This study was designed to evaluate the effect of ethanolic leaf extract of *Chromolaena odorata* on the immune system and spleen of Albino Wistar Rats. 41 adult male rats weighing between 110g -140g were used for this study. LD_{50} was carried out using 13 rats while 28 rats were used for the experiment proper. The rats used for the experiment proper were divided into four groups of 7 animals each. Group A served as control group and received animal feed and distilled water only, Group B received 1000mg/kg of the leaf extract and water, Group C received 3000mg/kg of the leaf extract and water, and Group D received 6000mg/kg of the leaf extract and water. The ethanolic leaf extract of *Chromolaena odorata* was administered 4-times per week for 13 weeks. The animals were killed using diethyl ether suffocation, blood samples were collected by ocular puncture for haematological analysis of RBC, WBC and differentials, Platelets, CD4 and CD8 cells count, the spleen and peyer's patches harvested, weighed and the spleen immediately fixed in 10% formol saline for histological analysis. Data was considered significant at $p<0.05$. Result showed a significant increase ($p<0.05$) in body weight in the groups when compared to the control group A. Peyer's patches had a significant decrease ($p<0.05$) in group B and C, while group D had a significant increase ($p>0.05$). Hemoglobin concentration, and PCV revealed a significant ($p<0.05$) decrease when compared to the control group. RBC result showed a significant ($p<0.05$) decrease when compared to group A. Platelet count revealed a significant ($p<0.05$) increase in the groups. CD4 and CD8 count also revealed a significant increase. Histopathological study showed reactive lymphoid follicles in group B, circumferential zone of necrosis around a disc shaped eosinophilic, foreign material was seen in group C, and group D had necrosis when compared to control group. In conclusion, the ethanolic leaf extract of *Chromolaena odorata* have toxic effect on hematological indices of RBC, PCV with no effect on immune cell functions of Cd4 and CD8 cell counts and white blood cells.

Key words: chromolaenaodorata, ethanolic extract, spleen, immune system

INTRODUCTION

"Chromolaenaodorata is known by many names including Siam weed, Christmas bush, devil weed and common floss flower. In Nigeria, it is commonly known as Ewe Awolowo, Siam weed, Elizabeth weed, Obirakara, Oloroohuru, independent weed and bienqua among the Ijaws in the Niger Delta region of Nigeria" (Ngozi and Osuji, 2014). "*Chromolaenaodorata* is a rapidly growing perennial herb. It is a multi-stemmed shrub that grows up to 2.5 m (100 inches) tall in open areas. It has soft stems but the base of the shrub is woody. In shady areas it becomes etiolated and behaves as a creeper, growing on other vegetations. It can then become up to 10 m (33 feet) tall" (Apori*et al*, 2000). The plant is hairy and glandular, and when crushed, the leaves emit a pungent, aromatic odor. The leaves are opposite, triangular to elliptical in shape, and have serrated edges.

"The plant is believed to possess healing potentials for wounds and treatment of pile ailment" (Egunjobi, 1969). "A decoction of the leaf is used as a cough remedy and as an ingredient with lemon grass and guava leaves for the treatment of malaria. Other folkloric medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory, diuretic tonic, antipyretic and heart tonic" (Vital and Windell, 2009). "The fresh leaves and extract of *Chromolaenaodorata* are traditional herbal treatment in some developing countries for burns, soft tissue wounds and skin infections" (Isirima and Siminialayi, 2018; Ikewuchi et al., 2021). "A formulation prepared from the aqueous extract of the leaves has been licensed for clinical use in Vietnam" (Ayyanar and Ignacimuth, 2009). "In Nigeria the local use of the leaf extracts of *Chromolaenaodorata*for sore throat and treatment of piles, burns and wounds have been documented" (Egunjobi, 1969). "It has also been reported to possess anti-inflammatory, astringent, diuretic and hepatropic activities" (Uyi et al., 2000)). "Chromolaena odorata is considered an invasive weed of field crops and natural environments in its introduced range" (King and Robinson, 1997). "It has been reported to be the most problematic invasive species within protected rainforests in Africa" (Struhsaker et al, 2005)

"The clinical effects of *Chromolaenaodorata* in which wound healing occurred, was attributed to the proliferation of fibroblast and endothelial cells" (Toan- Thang*et al*,2001).

"The polyphenolic extracts of the leaves showed antimicrobial activity against Staphylococcus aureus and Staphylococcus epidermidis"(Nurul*et al*, 2006). In the work by Ngono*et al*, (2006), "ethanol extracts of *Chromolaenaodorata* inhibited the in vitro growth of *Cryptococcus neoformans*, *Microsporiumgypseum, Trichophyton, Mentogrophyles and Trichophytonrubrum"*. Okigbo and Ajalie, (2005) reported that "leave extracts were inhibitory against four human pathogens such as *Bacillus aureus, Staphylococcus aureus, E coli* and *Salmonella typhi"*. "The dichloromethane-water extract of the plant exhibited significant anti-herpes simplex virus-1 and anti-malarial activity" (Ling et a*l*, 2007). Thoden*et al, (*2007) have also demonstrated profound nematicidal activity of the herb. The nitric oxide scavenging activity of the *Chromolaenaodorata*extract was demonstrated by (Alisi*et al*, 2008), in which "it was quantitatively determined by the total phenolic content, which shows that the extract contains an appreciable quantity of the phenolic compounds and these compounds might be responsible for the antioxidant potential of the extract". Jannah*et al*, 2006 in rats proved their efficacy as an antiulcer agent when used orally.This research is aimed at investigating the protective function of *Chromolaenaodorata* in haematological indices and immune system.

Materials and methods

Study Location

This research was carried out in the department of Anatomy Nnamdi Azikiwe University, Nnewi Campus.

Plant Identification and extraction

Fresh *Chromolaenaodorata* leaves were collected from a farm at Nnamdi Azikiwe University, Awka Campus. Identification of this plant was carried out in the Department of Botany Nnamdi Azikiwe University Awka and issued the number NAU/BOT/19/02435. The fresh leaves of *Chromolaenaodorata* plant were shade dried under room temperature at (29-35°C) for 2 weeks, after which the leaves were pulverized into coarse form with Accestor high-speed milling machine. 200g of the coarse form was then macerated in absolute ethanol for 24 hours and shaken intermittently. After that, the extract was filtered using Whatman's filter paper. The resulting ethanol extract was concentrated and evaporated to dryness using rotary evaporator at a temperature of 45˚C to avoid denaturation of the active ingredients.

The concentrated extract was stored in the refrigerator at 10˚C until use. This was done according to the method of Ugwu*et al*, 2013.

Phytochemical analysis of *Chromolaenaodorata*

Small sample of the leaf extract of *Chromolaenaodorata* was taken to Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Agulu Campus, Anambra State for qualitative phytochemical analysis using standard procedures as described by Sofowora, (1993) to test for the presence of tannin and saponin, Flavonoids, Anthocyanins, Quinines, Glycosides, Cardiac glycosides, Terpenoids, Phenols, Coumarins, Steroids, Alkaloids.

Animals

Forty-one (49) male Wistar rats weighing between 110-140g (21 for LD_{50} determination and 28 for the experiment proper) were used for the study. Animals were acclimatized for two (2) weeks in the animal house of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus. The Animals were maintained under standard laboratory conditions of light (12 hours), temperature and ventilation.

After acclimatization, animals were divided into 4 groups of 7animals each as follows:
Group A was the control group (received only animal feed and water), Group B received 1000mg/kg of the ethanolic leaf extract of *Chromolaenaododrata*, Group C received 3000mg/kg of the ethanolic leaf extract of *Chromolaenaododrata*, Group D received 6000mg/kg of the ethanolic leaf extract of *Chromolaenaododrata.* Extract were administered to the animals orally for 28 days after which the rats were sacrificed.

Acute Toxicity Study

The mean lethal dose (LD_{50}) test of *Chromoleanaodorata* leaves was carried out in the department of Physiology, Nnamdi Azikiwe University according to the method of Dietrich Lorke (1983). In the acute toxicity study, a total of 21 rats were used. They received the extract via oral route and it was carried out in two phases.Inphase 1 nine (9) rats were used and they were grouped into three (3) groups of three (3) rats each. Group 1 received 10mg/kg, group 2 received 100mg/kg, and Group 3 received 1000mg/kg. In phase II four (4) rats were used and they were grouped into four groups of three rats per group. Group 1 received 1200mg/kg, group 2 received 1600mg/kg, group 3 received 2900mg/kg, and group 4 received

5000mg/kg. The animals were monitored over a period of 24hrs for behavioural changes, food intake and mortality.The LD_{50} was calculated as follows

$LD_{50} = \sqrt{a \times b}$ where a = maximum dose with 0% mortality (5000mg/kg)

b= maximum dose with 100% mortality (Nil)

LD_{50} of *chromolaenaodorata*is found to be above 5000mg/kg.

Termination of experiment, Animal Sacrifice, Organ Harvesting and collection of Blood Samples

At the end of the experiment, (24hours after last administration), the rats were anesthetized in an enclosed chamberunder concentrated chloroform vapour.Blood samples were collected directly by ocular puncture into EDTA containers for full blood count, CD4 and CD8 test and plain serum bottle (for antioxidant test), in which the serum were separated by centrifugation and was stored in a refrigerator of temperature- 18c for biochemical analysis. Thereafter the animals were placed on the dissecting board, pinned to the board and dissecting set to harvest the spleen which was immediately weighed and fixed in 10% formal saline for histological studies using H & E method.

3.2.8 Statistical Analysis

Data obtained in this study was subjected to SPSS version 25; ANOVA was used to analyze the results of CD4, CD8, RBC, WBC, hemoglobin, Pack cell volume, Neutrophils, Lymphocytes, Relative spleen weight, and Payers' patches. T-test was used to compare the means of the body weights.Data was considered significant at $p<0.05$.

RESULTS

Physical Observation

After the administration of the ethanolic leaf extract of *Chromolaenaodorata* for the treated groups B, C and D the rats appeared to be calm, and showed some level of weakness and sluggish movement for about 3-5 minutes before having appetite for food and water, and afterwards become active again.

Results of Rats Body Weight

Table 1 shows the results of rat body weight changes. There was a significant ($p<0.05$) increase the weight of the animal in-group A when the initial weight was compared to the final weight. In the same vein, the results showed statistically significant increase in rat body weight in the experimental groups B and C when the final weights are compared to the initial weights. For group D however, although there was weight increase in final weight compared to the initial weight, the increase was not statistically significant.

Table 1: The effect of Ethanolic leaf extract of *Chromolaenaodorata* on body weight of Wistar rats

Groups	Body weight (g)	MEAN ±SEM	Weight diff	P-VALUE
Group A	Initial	111.66 ±4.01	58.33	0.000*
	Final	170.00 ±5.16		
Group B	Initial	122.00 ±5.83	52.00	
	Final	174.00 ±2.44		0.000*
Group C	Initial	113.33 ±3.33	50.00	
	Final	163.33 ±8.81		0.049*
Group D	Initial	145.00 ±8.66	32.50	
	Final	177.50 ±13.14		0.219

Data were analysed using Student dependent T-test and data were considered significant at P<0.05. *P<0.05 means significant, and P>0.05 means not significant

Table 2 shows the result of the effect of the extract on the weight of the spleen. It shows a statistically non-significant lower ($p>0.05$) relative spleen weight in the experimental groups B, C and D in a dose dependent fashion when compared to the control group A.

Table 2: The effect of Ethanolic leaf extract of *Chromolaenaodorata*on the relative spleen weight

Groups	MEAN ± SEM	P-VALUE
Group A (control)	0.44 ± 0.02	

Group B (Low Dose)	0.41 ± 0.03	0.656
Group C (Medium Dose)	0.46 ± 0.06	0.738
	0.39 ± 0.05	
Group D (High Dose)		0.461

Data were analysed using Student dependent T-test and data were considered significant at P<0.05. *P<0.05 means significant, and P>0.05 means not significant

Table 3 show the result of effect of ethanolic leaf extract of *Chromolaenaodorata*on the CD4 and CD8 counts. The result shows a statistically non-significant increase (*p>0.05*) in the mean CD4 count in groups B, C, and D when compared to the control group A,but the CD8 count showed a non-significant decrease (*p>0.05*) in-group B, C, and D when compared to group A.

Table 3: The effect of Ethanolic leaf extract of *Chromolaenaodorata*on the CD4 and CD8 counts

Immune parameters	Groups	MEAN ±SEM	P-VALUE
CD4 (cells/U)	Group A (control)	4.25 ±0.25	
	Group B (Low Dose)	5.62 ±0.23	0.135
	Group C (Medium Dose)	5.12 ±1.08	0.327
	Group D (High Dose)	5.00 ±0.40	0.399
CD8 (cells/mm^3)	Group A (control)	20.50 ±0.64	
	Group B (Low Dose)	17.50 ±1.04	0.339
	Group C (Medium Dose)	17.00 ±3.88	1.000
	Group D (High Dose)	16.75 ±1.25	0.237

Table 4 shows the effect of ethanolic leaf extract of *Chromolaenaodorata*on Haemoglobin concentration, Pack cell volume and Red blood cells.

Result from table 4 showed a significant decrease ($p<0.05$) in haemoglobin concentration in-group B, C, and D when compared to the control group A. Pack cell volume and red blood cell count showed significant decreases ($p<0.05$) in the experimental groups B, C, and D when compared to the control group A.

Table 4:The effect of Ethanolic leaf extract of *Chromolaenaodorata*on Haemoglobin concentration, Pack cell volume, and Red blood cells.

Parameter		MEAN ±SEM	P-VALUE
Hemoglobin	Group A (control)	11.40 ±0.34	
Concentration	Group B (Low Dose)	10.25 ±0.06	0.014*
(g/dl)	Group C (Medium Dose)	10.75 ±0.43	0.030*
	Group D (High Dose)	8.45 ±0.64	0.000*
Pack Cell Volume	Group A (control)	35.22 ±0.80	
(%)			
	Group B (Low Dose)	30.32 ±0.12	0.016*
	Group C (Medium Dose)	32.22 ±10.12	0.014*
	Group D (High Dose)	20.75 ±2.06	0.000*
Red Blood Cell	Group A (control)	6.43 ±0.20	
(x10 ^12/L)	Group B (Low Dose)	6.17 ±0.20	0.003*
	Group C (Medium Dose)	6.16 ±0.16	0.006*
	Group D (High Dose)	4.86 ±0.06	0.000*

Result from table 5 shows a significant decrease ($p<0.05$) in the white blood cell level in group B, C, and D when compared to group A. Result for Platelet count showed a significant ($p<0.05$) increase in group B, C, and D when compared to group A.

Table 5: The effect of Ethanolic leaf extract of *Chromolaenaodorata*on the white blood cell and platelet Count

Immune parameters Groups	MEAN ±SEM	P-VALUE

White Blood Cell (x10^3/L)	Group A (control)	14.15 ±0.41	
	Group B (Low Dose)	8.75 ±1.00	0.000*
	Group C (Medium Dose)	9.20 ±0.38	0.000*
	Group D (High Dose)	7.47 ±0.16	0.000*
Platelet count (x10^12/L)	Group A (control)	503.50 ±11.90	
	Group B (Low Dose)	630.50 ±18.98	0.000*
	Group C (Medium Dose)	581.50 ±14.36	0.004*
	Group D (High Dose)	553.50 ±16.94	0.045*

Table 6 shows the effect of Ethanolic leaf extract of *Chromolaenaodorata*on Neutrophils, Lymphocytes, and MID in Wistar rats. Result from table 6 show a non-significant decrease (*p<0.05*) in Neutrophil level in group B and C, while there was a significant (*p<0.05*) increase in group D when compared to group A. Result of Lymphocyte showed a significant (*p>0.05*) increase in-group B, but significant (*p>0.05*) decreases ingroups C and D when compared to group A.Result for MID showed a non-significant (*p>0.05*) decrease in-group B, C, and D when compared to group A.

Table 6: The effect of Ethanolic leaf extract of *Chromolaenaodorata*on Neutrophils, Lymphocytes, and MID

Parameter		MEAN ±SEM	P-VALUE
Neutrophils (%)	Group A (control)	13.40 ±0.38	
	Group B (Low Dose)	10.77 ±2.98	0.424
	Group C (Medium Dose)	13.37 ±1.98	0.994
	Group D (High Dose)	22.05 ±2.67	0.018*
Lymphocyte (%)	Group A (control)	84.30 ±0.55	
	Group B (Low Dose)	88.50 ±3.04	0.285

	Group C (Medium Dose)	82.27 ±3.30	0.603
	Group D (High Dose)	74.95 ±2.88	0.030*
MID (%)	Group A (control)	2.41 ±0.26	
	Group B (Low Dose)	1.92 ±0.39	0.506
	Group C (Medium Dose)	2.60 ±0.83	0.778
	Group D (High Dose)	2.35 ±0.18	0.350

Table 7 shows the effect of Ethanolic leaf extract of *Chromola enaodorata*on Glutathione Level. The result shows a non-significant (p>0.05) increase in the Glutathione level in groups B, C, and D when compared to group A.

Table 7: The effect of Ethanolic leaf extract of *Chromola enaodorata*on Glutathione Level

		MEAN	±SEM	P-VALUE
Glutathione	Group A (control)	740.50	±32.71	
Level	Group B (Low Dose)	851.00	±35.83	0.565
(µmol/g)	Group C (Medium Dose)	1062.50	±42.69	0.110
	Group D (High Dose)	862.50	±256.07	0.526

RESULT OF HISTOLOGICAL STUDIES AND histochemistry

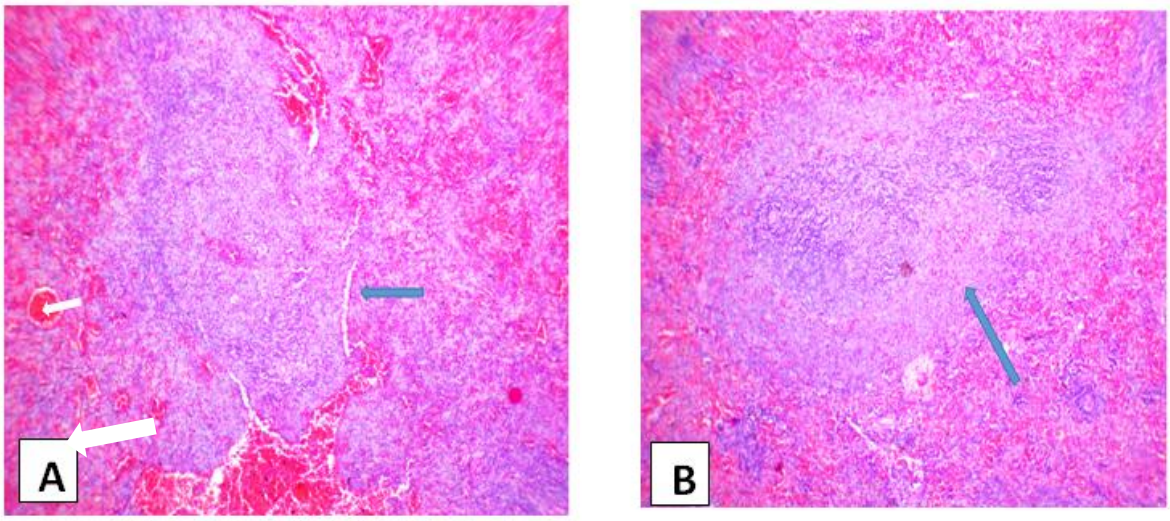

Plate 1: Photomicrograph section of spleen from rats in group A showing reactive lymphoid follicle (secondary follicle) (blue arrow), with the red and white pulp been intact (white arrow). H & E (X100). B shows the spleen of rats in group B administered with 1000mg/kg of *Chromlaenaodorata)* showing reactive lymphoid follicle (blue arrow). H & E (X100).

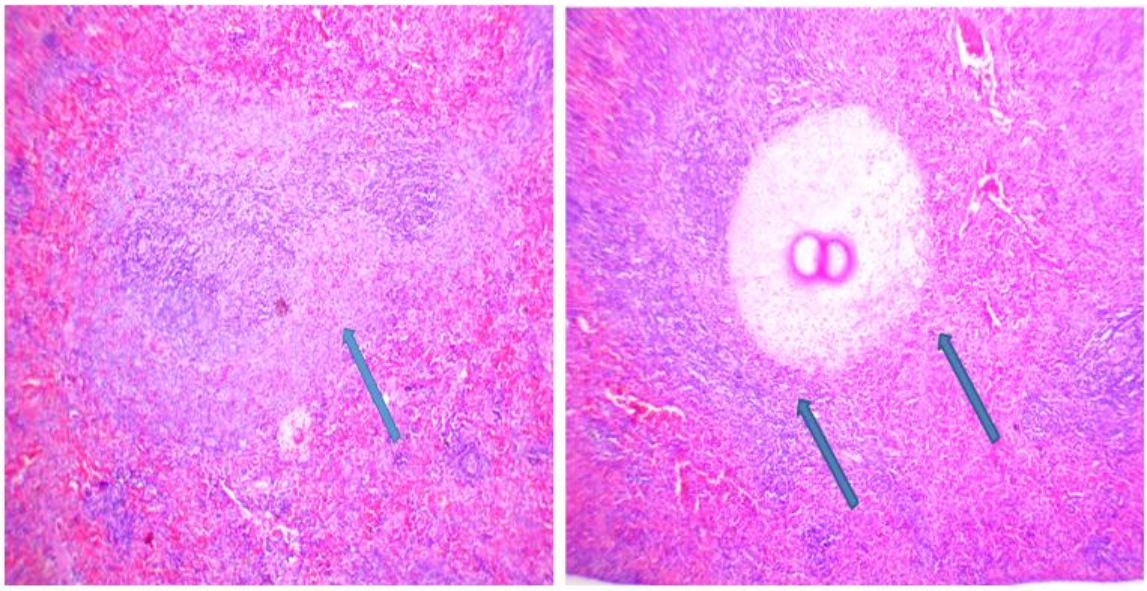

Plate 2: Photomicrograph of rat spleen in groups C and D showing (Group C)a well-defined circumferential zone of necrosis around a disc shaped eosinophilic foreign material (Blue arrows) and (Group D) Administered with 6000mg/kg of *Chromolaenaodorata*) showing disc shaped necrotic area (Blue arrows). H & E (X100).

DISCUSSION

The findings from this present study as shown in table 1 revealed a significant ($p<0.05$) increase in the body weight in group B, C and D group when compared to group A the control group. However, the precise mechanism of action for the significant growth rate was atrributed to high crude fiber and protein content present in *Chromolaenaodorata*. This is in line with Imaseun*et al*, (2017) that reported "a significant ($p<0.05$) increase in growth rate and feed intake following administration of *Chromolaenaodorata* in rabbits". And that of Clement *et al*, (2008) that reported "a significant increase in the body weight following aqueous extract of *Chromolaenaodorata* leaves".

Findings from table 2 revealed a non-significant ($p>0.05$) difference in the relative spleen weight in group B, C, and D when compared to control group A; with group B and D showed showing decrease, and group C had an increase. The reason for such occurrence is not well understood, but suggesting the presence of tannin, which has the potency of causing mild toxicity based on the spleen, causing infiltration and necrosis. This study contradicts the

findings of Isirima and Siminialayl (2018) that reported significant ($p<0.05$) changes in the spleen weight. Clement *et al.,* (2008) has similarity with this findings, which revealed a non-significant differences in the liver, kidney, and heart weight in the treated groups when compared to control following administration of aqueous extract of *Chromolaenaodorata.* Findings from this study revealed the Payer's patches weight showed a significant ($p<0.05$) difference in-group B and C when compared to group A although, group D had a non-significant ($p>0.05$) difference when compared to group A. The mechanism of action for the significant difference can be attributed to the presence of phytochemicals (flavonoids), which can cause an increase or decrease in the areas of payer's patches in the small intestine, thus increasing rate of absorption.

Findings from this present study showed a non-significant ($p>0.05$) difference in the CD4 and CD8 levels in-group B, C, and D when compared to control group A. The reason for this is well understood as a result of its nutritive and phytochemical contents. This corresponds with the report of Nudo and Catap (2017) who revealed in their study that the *Chromolaenaodorata*does not affect the CD4 and CD8 cell counts.

Findings from this study revealed showed a significant decrease ($p<0.05$) in haemoglobin concentration in group B, C, and D when compared to group A. Pack cell volume result showed a significant decrease ($p<0.05$) in-group B, C, and D when compared to group A. Red blood cell result showed a significant decrease ($p<0.05$) in-group B and C, and D when compared to group A. The precise mechanism of action for the significant reduction in RBC, hemoglobin, and pack cell volume is not fully understood. The reduction in RBC, hemoglobin, and pack cell volume could be attributed to the presence of tannin that has hemotoxic activity on RBC, haemoglobin, and pack cell volume causing a depletion in the production of these components of blood cell. The study of Henshaw*et al.,* (2017) contradicts the report of this study, and their findings showed a significant ($p<0.05$) increase in hemoglobin, and pack cell volume, following administration of aqueous and ethanolic extraction of *Chromolaenaodorata,* and gave their reasons as saponin been the key regulator of haematopoiesis. This study contradicts the findings of Nwakpa*et al.,* (2013) who reported a significant increase in RBC, hemoglobin, and pack cell volume following administration of *Chromolaenaodorata* is attributed to high anti-oxidants activity of flavonoids, which caused a reversing bone marrow depression with attendant improvement in erythrocyte membrane

stability, thus reducing haemolysis. This study agrees with the report of Imasuen*et al*, (2017), which showed a significant decline in RBC, at higher dose (20% and 30%) of *Chromolaenaodorata* leaf meal feed in rabbit.

Findings in this study showed that the ethanolic leaf extract of *Chromolaenaodorata* caused a significant increase in white blood cell count in group B, C, and D when compared to the control group. The mechanism of action why this increase occurred is because of the presence of Saponin paradox, when administered (Cheeke, 1971). This study agrees with the report of Imasuen, *et al*, (2017), which showed a significant decline in white blood cell at higher dose (20% and 30%) of *Chromolaenaodorata* leaf meal feed in rabbit. "Also, it revealed a significant ($p<0.05$) increase in platelet count, the precise mechanism of action is because of the presence of flavonoids which possess hyper stimulation of hematopoietic regulatory elements which regulates proliferation" (Yakubu and Afoloayan, 2009). This study agrees with the findings of Henshaw *et al*, 2017) who revealed a significant rise in platelet count at the 75mg/kg aqueous extracts showing improved platelet quality, hence its possible role in haemostatic as earlier reported by (Okoroiwu *et al*, 2016). "Increased mean platelet volume is an indication of larger and more reactive platelets resulting in increased platelet turnover. Larger platelets are more adhesive and tend to aggregate more than small platelets and contain more secretary granules" (Slavka *et al*, 2011). This study agrees with Isirima and Siminialayi (2018) who reported a significant increase in platelet count in methanol leaf extract of *Chromolaena odorata* following hematoxicity induced by *Salmonella typhi* in Wistar Rats.

Findings from this study showed a significant ($p<0.05$) increase in neutrophil levels in group D, with a non-significant ($p>0.05$) difference group B and C when compared to group A. The significant ($p<0.05$) increase in neutrophil count in group D, could possibly result from the higher quantity of flavonoids activities present in the plant extract. This agrees with the findings of Isirima and Siminialayi (2018) who reported "a significant increase in neutrophil levels in methanol leaf extract of *Chromolaena odorata* following hematoxicity induced by *Salmonella typhi* in Wistar Rats".

This study revealed also a significant ($p<0.05$) decrease in lymphocytes counting in group D, which was administered the highest dose. The reason for this decrease was suggested to be saponin haemolytic toxins, which have the potency of causing a decrease in lymphocyte levels. This study contradicts the report of Isirima and Siminialayi (2018) who reported a significant increase in 10%*Chromolaena odorata* leaf meal treatment group, and was attributed also to the presence of saponin. However, this study agrees with the same report of Isirima and Siminialayi (2018) who reported a significant increase in 20% and 30% of COLM treatment group.

The result of this study revealed a significant ($p>0.05$) increase in the Glutathione level in group B, C, and D when compared to group A. However, this study contradicts the report of Alisi*et al*, (2011) who reported a significant decrease in gluathione level following administration of ethanolic*Chromolaena odorata*-treated rabbits after CCL_4 toxicity, and gave their reasons that *Chromolaenaodorata* leaf extract has high content phenolic compounds, which reduces the damaged caused by CCL4 toxicity in increasing glutathione activity.

Findings from histopathological study showed reactive lymphoid follicles in group B, circumferential zone of necrosis around a disc shaped eosinophilic foreign material was seen in group C, and group D had necrosis when compared to control group, which had normal spleen with the red and white pulp been intact. The reason for these reactions noted in the different groups of the spleen is due to the saponin present in the *Chromolaena odorata*. This study agrees with the report of Isirima and Siminialayi (2018) who revealed "a significant change in the histology of the spleen, following administration of the *Chromolaena odorata* in Wistar Rats"

Conclusion

The results obtained from this study showed that the ethanolic extract of *Chromolaena odorata* leaf caused a reduction in Red blood cell, haemoglobin and Pack cell volume, which signifies anaemic condition. However, the white blood cell and lymphocytes activities showed an increase in their activities, signifying an increase in immune function. The result from this study do not affect the immune cell functions of CD4 and CD8 when administered. However, the extract showed necrosis in the spleen, which means that, the extract is toxic to spleen and can cause depletion of haematological functions.It is recommended that the

ethanolic extract of *Chromolaena odorata* should not be consumed at high rate since it shows signs of toxicity on some haematological parameters.

Ethical approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences Research Ethics Committee, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

REFERENCES

Alisi, C.S, Onyeze, G.O.C, Ojiako, O.A.,and Osuagwa, C.G. (2011). Evaluation of the Protective Potential of Chromolaenaodorata Linn. Extract on Carbon Tetrachloride-Induced Oxidative Liver Damage. *International Journal of Biochemistry Research & Review*, 1(3): 69-81.

King L., & Robinson, R. (1997). "Siam weed or chromolaena (Chromolaenaodorata)" *Journal of Weed Management Guide,* 3(2): 17-34

Ngono N, A.; Ebelle E, R.; Ndifor, F. Biyiti, L.;AmvamZollo, P.H. and Bouchet, P. (2006). Antifungal Activity of Chromolaenaodorata (L.)King and Robinson (Asteraceae) of Cameroon international of ethnopharmcognosy.

Nurul .A.H., Mamati.A.S, Effendi A. W. M. Hussin., Z. M and Sayed.M.Z.H. (2006). Extract of the leaves of Chromolaenaodorata on gram-positive bacteria, journal of ethnopharmacology. Pp51

Okigbo, R. N and Ajalie, A. N. (2005). Inhibition of some human pathogens with tropical plants extracts (Chromolaenaodorata and Citrus aurantifolia and some antibiotic). International Journal of Molecular Medicine and Advance Sciences, 1 (1) pp 34-40.

Struhsaker, T.T.,Struhsaker, P.J.,andSiex, K.S. (2005). "Conserving Africa's rain forests: problems in protected areas and possible solutions" (PDF).*BiologicalConservation*, 123 (1): 45–54.

Toan-Thang,.P., Lingzhi W., Patrick S., Renee J. G., Sui-Yung.C., and Lee, S. T. (2001). Phenolic Compounds of Chromolaenaodorata Protects Cultured Skin Cells from Oxidative Skin Cells from Damage: Implication for Cutaneous Wound healing", Boiling Pharmacological Bulletin, vol.24, 1373-1379

Uyi OO, Ekhator F, Ikuenobe CE, Borokini TI, Aigbokhan EI, Egbon IN, Adebayo AR, Igbinosa IB, Okeke CO, Igbinosa EO, Omokhua GA. (2014). Chromolaenaodorata invasion in Nigeria*: A case for coordinated biologicalcontrol Management of Biological Invasions, 5(4): 377–393.*

Vinay Kumar; Abul K. Abbas; Nelson Fausto; Richard N. Mitchell (2007*)."*The human erythrocyte*." Robbins Basic Pathology (8th ed).* 45 (2): 195–200.*).*

Vital P G and Windell L R. 2009.Antimicrobial activity and cytotoxicity of Chromolaenaodorata (L. f.)King and Robinson and Uncariaperrottetii (A. Rich) Merr.Extracts. Journal of Medicinal Plants Research; 3(7): 511-518.

Isirima, J. and Siminialayi, I. (2018) Effect of Chromolaena odorata Extracton Hematotoxicity and Spleen Histopathology Induced by Salmonella typhi in Wistar Rats. Pharmacology & Pharmacy, 9, 85-99. doi: 10.4236/pp.2018.94007.

Ikewuchi CC, Ikewuchi JC, Ifeanacho MO, Jack DP, Ikpe CN, Ehiosun S, Ajayi TB. Protective effect of aqueous leaf extracts of Chromolaena odorata and Tridax procumbens on doxorubicin-induced hepatotoxicity in Wistar rats. Porto Biomed J. 2021 Dec 3;6(6):e143. doi: 10.1097/j.pbj.0000000000000143. PMID: 34881354; PMCID: PMC8647896.