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EFFECTS OF SIAM WEED (*Chromolaena odorata*) LEAF EXTRACT ON HEMATOLOGICAL PARAMETERS AND LIPID PROFILE OF WISTAR ALBINO RATS (*Rattus norvegicus*)

[1]Zitte, Leelee Famii and [2]Mbagwu, Chiamaka Cynthia

[1,2]Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Choba, Port Harcourt, Rivers State.
Corresponding Author: Leelee.zitte@uniport.edu.ng +234-8037971401

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ABSTRACT

The study investigates the effects of Chromolaena odorata leaf extract on the liver and blood parameters of Wistar albino rats (Rattus norvegicus). The rats were divided into four groups of four rats per group. Group 1 (control) received only feed and normal saline, groups 2 , 3 and 4 were treated with, 400mg/kg bw, 800mg/kg bw, and 1600mg/kg bw of chromolaena odorata ethanol extract respectively, on a daily basis for 21 days. After the administration period, the animals were sacrificed and the blood and liver taken for analyses. The result showed that groups 1-4 high density lipoprotein(HDL) had mean values of 1.5±0.12 mmol/l, 1.17±0.28 mmol/l, 1.15±0.17 mmol/l, and 1.1±0.15 mmol/l respectively, while low density lipoprotein (LDL) had mean values of 0.20±0.08 mmol/l, 0.40±0.13 mmol/l, 0.30±0.20 mmol/l, and 0.27±0.14 mmol/l respectively. The triglyceride (TG) values were 4.92±0.20 mmol/l, 5.02±0.28 mmol/l, 5.72±0.24 mmol/l and 4.72±0.33 mmol/l for groups 1, 2, 3, and 4 respectively. There were no significant differences ($p>0.05$) in lipid profile when comparing the different groups and the control. However, the hematological parameters showed that the extract significantly reduced the red blood cell (RBC) from 6. ± 016 x 10^{12} cells /l in the control to 5.35±0.32 x10^{12} cells /l , 4.93± 0.23x10^{12} cells /l and 4.87±0.24 x10^{12} cells /l in groups 2,3,and 4 respectively. The packed cell volume (PCV) was also significantly reduced from 46.8 ±1.2 % in the control to 43.25± 0.96%, 41.00±1.16 % and 39.40± 0.57% in groups 2, 3 and 3 respectively. The hemoglobin concentration was also significantly reduced from 15.60±0.43 g/dl in the control to 14.50±0.34 g/dl, 13.70±0.41 g/dl, and 13.10±0.17 g/dl in groups 2, 3 and 4 respectively. The platelet count was reduced from 265± 38.73 x10^{9} cells /l in the control to 197.5±12.59x10^{9} cells /l 176.7±30.6x10^{9} cells /l in groups 2 and 4. Group 3 was not significantly different from the control. The WBC also had a significant reduction from 7.65±0.89x10^{9} cells /l in the control to 4.9±1.17c10^{9} cells /l, 5.66±0.74x10^{9} cells/l and 5.57±1.88x10^{9} cells /l. The neutrophil and lymphocytes were however not different significantly between the treatments and the control. This suggests that Chromolaena odorata leaf extract significantly affects the heamatological parameters but does not significantly affect the lipid profile.

Key words: Chromolaena odorata , hematological parameters , lipid profile.

INTRODUCTION

There have been several researches on medicinal plants and their bioactive derivatives which are potent resources for pharmacological products. Findings in the use of herbal products for medicinal purposes have contributed immensely towards the discovery and manufacture of

synthetic therapeutic products. Chromolaena odorata (siam weed), an invasive perennial shrub that grows in the tropical and subtropical region, is one of the plants known for its toxic and therapeutic properties. For example, the leaves are toxic to cattle (Sa Ije, *et al*, 2008). It is also used for therapeutic purposes because of its antiprotozoal, antitrypanosomial and antibacterial characteristics. The leaves are used against skin infections, to stop bleeding and for wound healing (Alisi *etal.* 2011a)

It is distributed within the South and Central America, Africa and tropical Asia (Muniappa and Muratani 2006). It is known to prevent the regeneration of other crops, hence, affecting species diversity. Alisi *et al*, (2011b) reported that aqueous extract of *C. odorata* leaf has a tremendous antioxidant and antihyperglycemic activities, which are great assets in the management of diabetes. This was similar to the findings of *Ijioma et al*, (2014) who separately reported that ethanol extract of *C. odorata leaf* has hypoglycemic, hematologic and lipid profile effects on alloxan induced diabetic rats. Hataichanok *et al,* (2013) reported that siam extract promotes Balb/c 3T3 fibroblast cell migration and proliferation. They also observed that, subsequently, the heme oxyginase- 1 (HO-1), an accelerating wound healing enzyme, was increased at the transcriptional and translocational levels by siam weed extract treatment.

Alisi *et al*, (2012) concluded that the leaf extract has hepatoprotective potentials against carbon tetrachloride induced oxidative damage. The leaves are also seen to protect human dermal fibroblast and epidermal kerafinocytes against hydrogen peroxide and hypoxanthine–xanthinenoxidase induced damage (Phang *et al*, 2001). Nwankpa *et al*, (2012) reported that the ethanolic extract of *C. odorata* leaf has an oxidative potential against oxidative damage induced by infection of *Salmonella typhi* in rats. Nuruihuda *et al*, (2004) in their studies, observed that the extract can be considered as a potential bactericidal agent against gram negative bacteria. A number of studies also show that the extract inhibits the growth of *Pseudomonas aeroginosa, E. coli, Staphylococcus aureus* (Irobi, 2006). In another development, Pierangeli *et al*, (2009) suggested the potency of the extract as antibacterial and anti-protozoa sources; and that it is of high probability that this potential could be developed into antimicrobial drugs if specific compounds are be isolated and purified.

Natheer *et al*, (2012) carried out a research on antibacterial activity of the leaf extract, and that the extract was useful in the treatment of infectious diseases and in the development of novel chemotherapeutic agent. In traditional medicine, a decoction of the leaf was used as an ingredient with lemon grass and guava leaves in the treatment of malaria (Doss et al, 2011). *C odorata* leaves were found to possess antibacterial (Lavanya and Brahmaprakash, 2011), anti-inflammatory activity (Owoyele *et al*, 2005, Ayyana and Iguacimuthu, 2009, Pauillac *et al*, 2009) when the fresh leaves are ground into a paste and applied tropically on affected places to heal wounds (Kilani, 2006). In folk medicine, the aqueous leaf extract of the plant was used as antiseptic for wound dressing.

Kigigha and Douye, (2013) investigated the activity of *C. odorata* on enteric and superficial etiologic bacteria agents. Their result shows that the ethanol extract is significantly less inhibitory on E. coli when compared with 1% ampiclox. Asomugha *et al*, (2014) investigated the hepatotoxic effect of aqueous extract of the leaf on male albino rats and concluded that it has the ability to produce toxicity of the liver at high dose level and they suggested that

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human exposure for a long time needs to be closely monitored.

Anti- Cholesterol Effect of *Chromolaena odorata*

Ikewuchi *and* Ikewuchi, (2011) from their studies, reported a possible anticholesterolemic and protective mechanism of aqueous extract of the leaves of *Chromolaena odorata* against the development of atherosclerosis and coronary heart disease as well as the dyslipidemic conditions that characterize obesity, hypertension and diabetes mellitus. This was also affirmed by the report of Ghiadoni and Salvetti (2007). On the dyslipidemic condition. Owoyele *et al.*, (2005) opined that the leaf of *C. odorata* has the ability to reduce chronic inflammation. It was observed to have less antiinflammatory activity and more analgesic activities than ethanol extract (Owoyele *et al,* 2006). This was based on the flavonoid content (Owoyele *et al*,2008).

Nwankpa *et al.*, (2013) studied the effect of *C. odorata* leaf extract on hematological profiles of *Salmonella typhi* infested Wistar rats. Their result showed that ethanol extract of *C odorata* significantly increased the red blood cell (RBC) count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) thereby reducing and ameliorating the anaemic condition induced by *Salmonella typhi* infection. It was also observed to have improvement in erythrocyte membrane stability thus reducing haemolysis due to its antioxidant potential (Naaz *et al,* 2007, Nwankpa *et al*, 2012). Alisi *et al*., (2012) also showed that *C. odorata* leaf extract possess antimicrobial activity which inhibits the growth of *Salmonella typhi* in cells. Alkaloids in C odorata are seen to significantly decrease (p<0.05) the testes–body weight ratio; concentrations of testicular total protein, glycogen, sialic acid, and cholesterol; and the activities of gamma-glutamyl transferase, acid phosphatase, and alkaline phosphatase in the body.

MATERIALS AND METHODS

A total of sixteen (16) adult albino rats comprising of 8 male and 8 female weighing between 160 and 200g were used for this study. The rats were kept in the Animal House of the Departments of Anatomy and Physiology, University of Port Harcourt. They were housed under standard laboratory conditions with a 12 hour day/night cycle with free access to feed and water. They were also allowed to acclimatize to laboratory conditions for one week before the commencement of the experiment.

Leaves of *Chromolaena odorata* were plucked from Alakahia, ObioAkpor Local Government Area, Rivers State. The leaves were air-dried and ground into powder and 100% ethanol was used to extract the active ingredient from the leaves and the extract was administered orally to the albino rats using syringe and oral Canulla in different doses of 400mg/kgbw/day, 800mg/kgbw/day and 1600mg/kgbw/day for groups 2 to 4 over a period of four weeks, while group one (control) was treated with normal saline solution over the same period.

Blood was collected through the tail vein into ethylene diaminetetraacetic acid (EDTA) bottles for blood cell analysis. The animals were anaesthetized in a chloroform before they were sacrificed and dissected. Their blood samples were collected into different universal bottles for lipid and hematological analysis. The analyses were performed at Lively Stone Medical. Diagnostic Laboratory, Choba, Rivers State.

Total cholesterol and triglyceride analyses were conducted using three test tubes labeled as test sample, standard (200mg/dl cholesterol) and blank. Then 1ml of reagent was pipette into all the test tubes using distilled water as blank. The contents were mixed and incubated at $25^{O}C$ for 10 minutes. The absorbent of the sample and the standard were measured against the blank at the wavelength of 540nm.

Cholesterol concentration (mg/dl)= $\frac{\text{absorbance of sample}}{\text{absorbance of standard}}\ x\ 200$

The triglyceride was analyzed using similar method but with triglyceride reagent and standard. The triglyceride was measured against the blank at an absorbance level of 500nm.

Triglyceride conc.(mmol/l) = $\frac{\text{absorbance of sample}}{\text{absorbance of standard}}\ x\ conc.of\ standard$ (mmol/l)

During high density lipoprotein (HDL) analysis, Eighty mililitre (80ml) of the reagent (Phosphotengstic acid (0.55mmol/l) and magnesium chloride (22mmol/l)) was diluted with 20ml of distilled water. With the aid of a micro pipette, 200ul of the sample and the standard were pipetted into centrifuge tubes and 500ul of the diluted precipitant (the reagent) was added. The content was mixed and allowed to stand for ten minutes then centrifuge 4000 rpm for ten minutes to precipitate other lipid fractions like low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicron, after that the supernatant was separated into three different test tubes containing 50ul of distilled water, standard supernatant, and sample supernatant respectively, 500ul of HDL Reagent was added. The content was mixed for 10 minutes at room temperature and the absorbance determined with a wavelength of 500nm the result was measured against the blank.

HDL= $\frac{\text{sample}}{\text{standard !}}$ x concentration of standard. (mmol/l)

Hematological analysis

Blood corpuscles were determined using the Neubauer manual chamber, with the RBC, WBC and differential counts analyzed separately. The PCV analysis was done by centrifuging a blood sample in wintrobe tube and the PVC read from the graduated wintrobe tube. MCV MCH and MCHC were separately calculated

Statistical Analysis

The data obtained was analyzed by ANOVA with a probability level of 5% ($P \leq 0.05$),

RESULTS

Effects of the siam weed leaf extract on Lipid Parameters

Treatment of albino rats for 21 days with *Chromolaena odorata* (3ml) caused no significant difference ($P>0.05$) in the total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein values when the values of the different groups were compared with the respective controls. (Table 1).

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Table 1: Result of effect of *C. odorata* extract on Lipid parameters

Parameters / Treatment	No. of Animals	TC (mmol/l)	TG (mmol/l)	HDL (mmol/l)	LDL (mmol/l)
T1 (control)	4	3.63 ± 0.40	4.93 ± 0.21	1.5 ± 0.13	0.21 ± 0.08
T2(400mg/kg)	4	3.75 ± 0.33	5.02 ± 0.29	1.15 ± 0.02	0.40 ± 0.13
T3 (800mg/kg)	4	3.85 ± 0.36	5.73 ± 0.25	1.15 ± 0.17	0.31 ± 0.20
T4 (1600mg/kg)	4	3.13 ± 0.37	4.73 ± 0.33	1.1 ± 0.15	0.28 ± 0.14

Each value represents the mean ± S.E.M (n =4)

Effects of *Chromolaena odorata* leaf extract on Haematological parameters of albino rats

Administration of different doses of *Chromolaena odorata* to different groups of albino rats for 21 days expressed some degrees of significant differences ($p \leq 0.05$) in the PCV, PLT, RBC and WBC amongst the different treatment groups and with the control (table 2). The treatment significantly ($p < 0.05$) reduced the RBC from 6.0± 0.16x10^{12} cells /l in the control to 5.35± .32x10^{12} cells /l in group two, 4.93 ± 0.23 x10^{12} cells /l in group three and 4.87 ± 0.24 x10^{12} cells /l in group four. The PCV was significantly ($p < 0.05$) reduced from 46.8 ± 1.26 % in the control to 43.25 ± 0.96 % in group two , 41 ± 1.16% in group three and 39.4 ± 0.57 % in group four. Hemoglobin level in groups one, two, three and four were 15.6±0.43 g/dl, 14.5±0.34g/dl, 13.7±0.41g/dl and 13.1± 0.17g/dl respectively with the treatment groups expressing significant difference amongst themselves and with the control. The platelet had a dose independent values of 265±38.73 x10^9 cells /l, 187.5±12.59x10^9 cells /l, 272.5 ± 5x10^9 cells /l and 176.7±30.6x10^9 cells /l for the control (group 1) groups two, three and four respectively. The extract also had an effect on the WBC of the animal by significantly decreasing ($p < 0.05$) the value from 7.65± 89x10^9 cells /l in the control to 4.9±1.17 x 10^9 cells /l 5.66 ±0.76 x10^9 cells /l and 5.57±1.88 x10^9 cells /l in groups two, three and four respectively. However, the neutrophils and lymphocytes did not indicate any significant difference ($P>0.05$) between the treatment groups and the control. (Table 2). In the mean corpuscular volume, (MCV) and mean corpuscular hemoglobin (MCH) group one result was significantly different from groups three and four but was not significantly different from group two which was seen also not to be significantly different from groups three and four. However, in the mean corpuscular hemoglobim concentration (MCHC) there was no significant difference ($P>0.05$) amongst all the groups (Table 3).

Table 2: Result of effects of *C. odorata* extract on some Blood parameters

Parameters / Treatments	**n**	**RBC (x10^{12} cells /l)**	**PCV (%)**	**HB (g/dl)**	**PLT (x10^9 cells /l)**	**WBC (x10^9 cells /l)**	**Neutrophil (%)**	**Lymphocytes (%)**
T1 (control)	4	6 ± 0.16^a	46.8 ± 1.26^a	15.6 ± 0.43^a	265 ± 38.73^a	7.65 ± 0.89^a	29.25 ± 2.99	69.25 ± 5.38
T2 (400mg/kg)	4	5.35 ± 0.32^b	43.25 ± 0.96^b	14.5 ± 0.34^b	197.5 ± 12.59^b	4.9 ± 1.17^b	33.25 ± 9.07	66.75 ± 9.07
T3 (800mg/kg)	4	4.93 ± 0.23^c	41 ± 1.16^c	13.7 ± 0.41^c	272.5 ± 5^a	5.66 ± 0.76^b	24.25 ± 4.34	75.75 ± 4.35
T4 (1600mg/kg)	4	4.87 ± 0.24^c	39.4 ± 0.57^c	13.1 ± 0.17^c	176.7 ± 30.6^b	5.57 ± 1.88^b	29.34 ± 5.13	70.67 ± 5.14

Values are presented in mean ± S.E.M (n= 4) $p < 0.05$. ^{a,b,c} means values in a column with the same alphabetical superscripts are not significantly different.

Table 3: Effects of C odorata extracts on MCV, MCH and MCHC contents

Parameters / **Treatments**	n	MCV ($X10^{-14}$ L/cell)	MCH ($X10^{-11}$ g/cell)	MCHC (g/dl)
T1 (control)	4	7.79 ± 0.02^{a}	2.6 ± 0.009^{a}	33.36 ± 0.02
T2 (400mg/kg)	4	8.10 ± 0.15^{ab}	2.7 ± 0.05^{ab}	33.35 ± 0.04
T3 (800mg/kg)	4	8.33 ± 0.09^{b}	$2.75.02^{b}$	33.10 ± 0.20
T4 (1600mg/kg)	4	8.20 ± 0.17^{b}	2.74 ± 0.07^{b}	33.41 ± 0.11

Values are presented in mean ± S.E.M (n= 4) $p < 0.05$. ^{a,b,c} means values in a column with the same alphabetical superscripts are not significantly different.

DISCUSSION

The result on the lipid profile indicated that the extract has no significant effect on the different lipid parameters. The extract will not be a good therapy for atherosclerosis and its associated lesion. The hematological analyses however showed significant differences in the different parameters studied, especially the RBC, Hb, PCV, WBC and PLT. These parameters expressed significant reductions in the results of the different groups when compared with the control. However, the result was not dose deperndent amongst the treatment groups.

This result agrees with the finding of Fasuyi *et al.,* (2005) who reported that inclusion of siam weed more than 5% in feed causes a decrease in blood parameters like RBC, PCV , Hemoglobin concentration{Hbc}, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV). However, the result was contrary to the findings of Okoroiwu *et al*., (2017) who evaluated the effects of crude extract , aqueous extract and ethanol extract of *Chromolaena odorata* on blood parameters and reported that the three extracts cause a significant increase in the blood parameters when compared with the control. The difference could be attributed to the fact that they used very low concentrations of the extract (75mg/kg, 160mg/kg and 300mg/kg bw)

It was also different from the work of Nwankpa *et al.*, (2013) which showed that ethanol extract of *C. odorata* significantly increased the level of RBC, HB, PCV, MCV, MCH and MCHC in salmonella typhi infected patients, hence reducing and ameliorating the anaemic condition induced by *Salmonella typhi* infection.

CONCLUSION

Chromolaena odorata has the potential to influence hematological parameters especially when in a high dose. Its effects on lipid profile, which was observed to be insignificantly different from the control, could be traced to the fact that *Rattus norvegicus* has a low lipid content which influences the results so observed.

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