

Evaluation of Hepatotoxicity Associated With Ethanolic Extract of *Chromolaena Odorata* in Wistar Rat Model

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Abstract: Background Of The Study: To evaluate the adverse effect(s) of varying concentrations of *Chromolaena odorata* extract on Bilirubin level and Histology of liver in Albino rats.

Materials and Methods: The design of the study was a prospective cohort study. The place and duration was at Ikot Offiong Ambai, in Akpabuyo Local Government Area and Department of Botany, University of Calabar, between April 2016 and May 2017. We enrolled 20 albino rats with average weight of 54.0 g into four groups of five rats each. The test groups (1, 2 and 3) were orally given 100, 150, &250 mg/kg of ethanolic extract of *Chromolaena odorata* respectively. The control group (4) was orally given distilled water. The experiment lasted for 12 weeks. The rats were sacrificed using chloroform. The liver tissues were harvested and fixed using 10 % neutral buffered formalin for histology analysis. The blood samples were collected and analyzed for total bilirubin (TB) and conjugated bilirubin (CB) using H-Valley's method.

Results: Of the 20 albino rats, 15 albino rats of the 3 test groups showed significant decrease in their mean total bilirubin ($5.6 \pm 0.08 \mu\text{mol/L}$, $7.8 \pm 0.14 \mu\text{mol/L}$, and $9.4 \pm 0.17 \mu\text{mol/L}$ for groups 1, 2 and 3 respectively) compared to the control group ($11.1 \pm 0.23 \mu\text{mol/L}$) ($P < 0.05$). Similar trend was observed with the conjugated bilirubin ($3.5 \pm 0.06 \mu\text{mol/L}$, $3.7 \pm 0.09 \mu\text{mol/L}$, and $4.7 \pm 0.07 \mu\text{mol/L}$ for test groups 1, 2 and 3 respectively compared to $6.1 \pm 0.15 \mu\text{mol/L}$ for control group with significant increase ($p < 0.05$). Pearson's correlation showed total bilirubin and conjugated bilirubin are significantly but negatively associated with toxicity grades for liver tissues of test groups.

Conclusion: This study has shown *Chromolaena odorata* when taken in high concentrations and for a long period is toxic to the liver.

Keywords: *Chromolaena odorata*, hepatotoxicity, bilirubin, liver, albino Wistar rats.

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I. Introduction

Chromolaena odorata also called Eupatorium odoratum originated from South America. It is now a popular weed seen in most parts of the tropical and subtropical regions. *C. odorata* is a perennial semi-woody shrub in the Asteraceae family [1] as illustrated in figure 1. It has different names in different regions. In Nigeria, it is called independent and Awolowo [2], "obirato" in Eastern Nigeria and triffid in northern Nigeria [3]. Other names of *C. odorata* include siam weed, Christmas bush, Jack in the bush, devil weed, cohoi (Vietnamese), rumput siam (Malaylam), rumput Golkar (Indonesian), Kesengesil (Guam), Herbe du Laos (French), Cariaguillo Santa Maria (Spanish) and Siam Kraut (German) [4].



Fig. 1. Leave of *Chromolaena odorata* [5]

C. odorata is a multi-stemmed shrub that can grow up to 2.5m tall. It is hairy and glandular. It also has soft stems with the woody base and behaves like creepers in shady areas, growing on other plants where it can grow as tall as 10m. When the plant is crushed, it gives off a pungent and aromatic odour. The leaves of *C. odorata* measures about 10cm x 5cm in its widest dimension. The plant produces leaf petioles that are 1 – 4 cm in length while the white to pale pink tubular flowers are panicles of 10 – 35 flowers that form at the ends of branches. *C. odorata* can produce 80,000 – 90,000 seeds that are wind pollinated. Although, long distance pollination may be possible by cling to fur, cloths, and machinery [6].

Phytochemical analysis by Nwinuka *et al.*, [7] revealed that nutritional components of *C. odorata* include; carbohydrate, protein, lipid, ash, water and a high concentration of fiber. The mineral element components observed by the researcher include calcium, sodium, potassium, iron, magnesium, manganese, zinc, copper and phosphorous. The bioactive ingredients contents of *C. odorata* include alkaloids, flavonoids, saponins, cyanogenic glycosides, tannins and phytic acid [8]. *C. odorata* leaf is important in several ways. It belongs to one of the wide range of plants commonly used as medicinal remedies. Its role as an herbal remedy has been attributed to the numerous bioactive components of the plant. The plant has been reported to be potent for treatment of many disease conditions. *C. odorata* is known to have anticonvulsant [9], antidiabetic [10,11], antifungal [12,13] and antidiarrheal qualities [14,15]. It is also known to have antibacterial, anthelmintic, analgesic and antioxidant [16], blood clotting and wound healing properties [1,17]. In most parts of Nigeria, the leaf of *C. odorata* is used for the skin infection and to arrest bleeding [17]. Apart from its medicinal values, *C. odorata* is used for the improvement of soil fertility [18]. It has been reported that it enhances the buildup of organic matter in the fallow system in tropical soil [19] and can be used as a pesticide [20, 21].

Chromolaena odorata has been reported to be toxic to cattle [6] due to its high nitrate level [17]. *C. odorata* can also cause an allergic reaction. It contains alkaloids (pyrrolizidine) which are known carcinogens [22]. Previous studies have revealed a toxic effect of *C. odorata*. Asomugha *et al.*, [8] had reported adverse effect of higher concentration (161.5, 323.0, 583.5, and 1077) mg/kg of *C. odorata* extract on the liver function of male Wistar rat, while Anyanwu *et al.* [23] reported its adverse effect on some kidney and epithelial mucosa of the intestine after administration of extract of *C. odorata* even at a lower concentration (100, 150 and 250) mg/kg. In this present study, the adverse effect of varying concentrations of an extract of *C. odorata* on the bilirubin and histology of the liver of albino rats was evaluated.

II. Materials And Methods

2.1. Plant material

C. odorata leaves were collected from the locality of Ikot Offiong Ambai, in Akpabuyo Local Government Area of Cross River State, Nigeria and were identified in the Department of Botany, University of Calabar, Nigeria. The leaves of *C. odorata* were dried under room temperature with further drying using an oven and ground to fine powder using an electric grinder. Approximately 250 g of the ground sample was weighed using an electronic weighing balance and dissolved in 1000 ml of absolute ethanol. This was properly

mixed and allowed to stand for 48 hours, after which it was filtered using Whatman No. 1 filter paper. The filtrate was concentrated by heating in a water bath at 40°C and the remaining solvent was removed in a rotary evaporator to produce crude extract called ethanolic extract of *C. odorata*.

2.2. Animals

Twenty (20) growing albino rats of both sexes with an average weight of 56 g were used in this study. The rats were bought from the Animal House, College of Medical Sciences, University of Calabar, Nigeria. The animals were housed in wire-gauze cages in a well-lit and adequately ventilated room, under standard environmental conditions (12 hours of light and 12 hours of dark cycle). They were allowed to acclimatize while being fed with standard laboratory animal chow and water *ad libitum* for 2 weeks.

2.3. Experimental groups

The 20 albino rats were divided into four groups of five rats each. Groups 1, 2 and 3 represented the test groups, while Group 4 was used as the control group. During the 6 weeks of the experiment, Groups 1, 2, and 3 were given 100 mg/kg, 150 mg/kg, and 250 mg/kg of an ethanolic extract of *C. odorata* orally respectively while the control animals (Group 4) were given only distilled water. The experiment continued for 12 weeks.,

2.4. Sample collection and preparation

At the end of the experiments, the rats were anesthetized with chloroform inhalation and then sacrificed. The blood samples were collected by heart puncture from each of the rats into well-labeled dry plain tubes for biochemical analysis, while the livers of the animals were harvested and immediately fixed in 10% neutral buffered formalin for histological studies. After 24 hours of fixation, the liver tissues were processed immediately via dehydration of tissue in ascending concentrations of alcohol, cleared in xylene, and infiltrated with paraffin wax before embedding with paraffin wax. Sections were cut at 4µm using rotary microtome and mounted on well labeled frosted end microscope slides and stained with hematoxylin and eosin. The blood samples were collected into already labeled dry plain tubes for bilirubin estimation. The blood samples were allowed to clot and spun at 12,000 g for 15 minutes. The serum of the blood samples was separated into different well-labeled dry plain tubes before analysis. H-Valley's method was used for Total and Conjugated bilirubin estimation.

III. Statistical Analysis

Data generated from laboratory assays were appropriately analysed for means, standard deviation and analysis of variance(ANOVA) using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Results were presented as average ± standard error of mean. Two sided $P = .05$ was considered statistically significant for ANOVA which was used to compare the mean values of the total and conjugated bilirubin of the test and control groups.

IV. Results

Figure 2 shows the mean values of total bilirubin (TB) for test groups (1, 2, 3) administered with varying concentrations of ethanolic extracts of *C. odorata* (100, 150, 250 mg/kg) and the control group administered with distilled water. The mean values of TB test groups and control were 5.6 ± 0.34 , 7.8 ± 0.66 , 9.4 ± 0.84 and 11.1 ± 0.92 µmol/L respectively. The results of TB decreased significantly in groups 1, 2 and 3 when compared with the control group ($P < 0.05$). However, increasing values of the parameters were observed as the concentrations of the extract were increasing. Post hoc analysis revealed a significant decrease ($P < 0.05$) in the TB value of group 1 administered with 100 mg/kg of *C. odorata* extract (5.6 µmol/l) when compared with the control group (11.1 µmol/l).

Figure 3 illustrates the mean values of conjugated bilirubin (CB) for test groups (1, 2, 3) administered with varying concentrations of the ethanolic extracts of *C. odorata* (100, 150, 250 mg/kg) and the control group (4) administered with distilled water. The mean values of CB were 3.5 ± 0.25 , 3.7 ± 0.55 , 4.7 ± 0.64 and 6.1 ± 1.57 µmol/L respectively. The results of CB decreased significantly in groups 1, 2 and 3 when compared with the control group ($P < 0.05$). Post hoc analysis revealed that values of CB in group 3 rats administered with 250 mg/kg of the extract increased (4.7 µmol/l) significantly when compared with those of groups 1 and 2 administered with 100 mg/kg and 150 mg/kg (3.5 and 3.7 µmol/l) respectively ($P < 0.05$).

Sections of liver tissues from control group in figures 4a and 4b showed prominent central vein (CV) with plates of hepatocytes radiating outward. The hepatocytes have abundant cytoplasm and round to oval basophilic nuclei. The separating sinusoidal spaces (SS) are dilated. The portal tracts contain the bile duct, hepatic artery and portal vein and mild inflammatory cells with intact limiting plate hepatocyte.

Sections of liver tissue from group 2 administered with 150 mg/kg of an ethanolic extract of *C. odorata* in figures 5a and 5b showing preserved architecture and a central vein that is congested, with prominent hepatocytes radiating outward. The hepatocytes are separated by dilated and congested sinusoidal spaces. The portal areas are dilated, containing marked inflammatory cellular infiltrate with piecemeal necrosis. This is suggestive of hepatotoxicity.

Sections of liver tissue from group 3 administered with 250 mg/kg of an ethanolic extract of *C. odorata* in figures 6a and 6b showing marked portal inflammation and patchy necrosis of hepatocytes. The sinusoidal spaces are dilated and congested. The central veins are also congested. Some of the hepatocytes have abundant eosinophilic cytoplasm and round to oval nuclei. This is suggestive of hepatotoxicity.

V. Figures

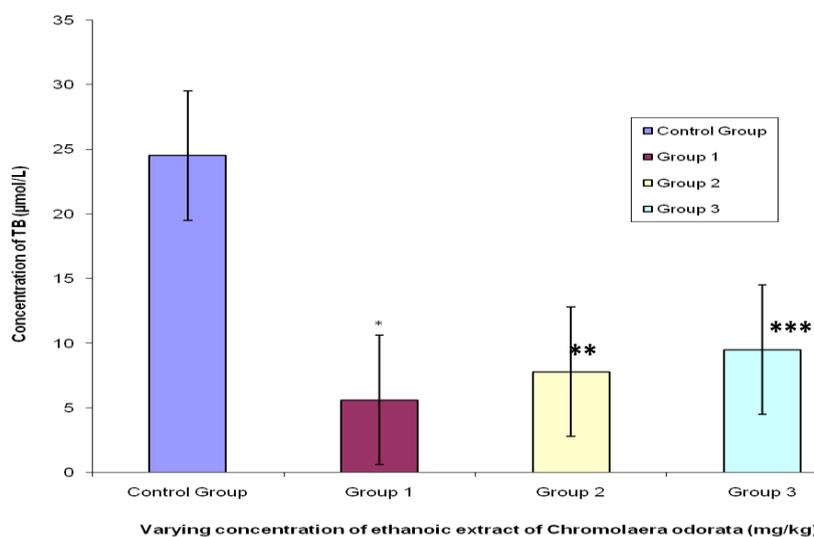


Fig. 2. Effect of different doses of *Chromolaena odorata* extract (100, 150, 250 mg/kg) on total bilirubin (TB) levels of Wister rats (p<0.05)

*significant as to control group

** = significant as to control group

*** = significant as to control group and Group 1

Mean ± S.E.M = Mean values ± Standard error of mean

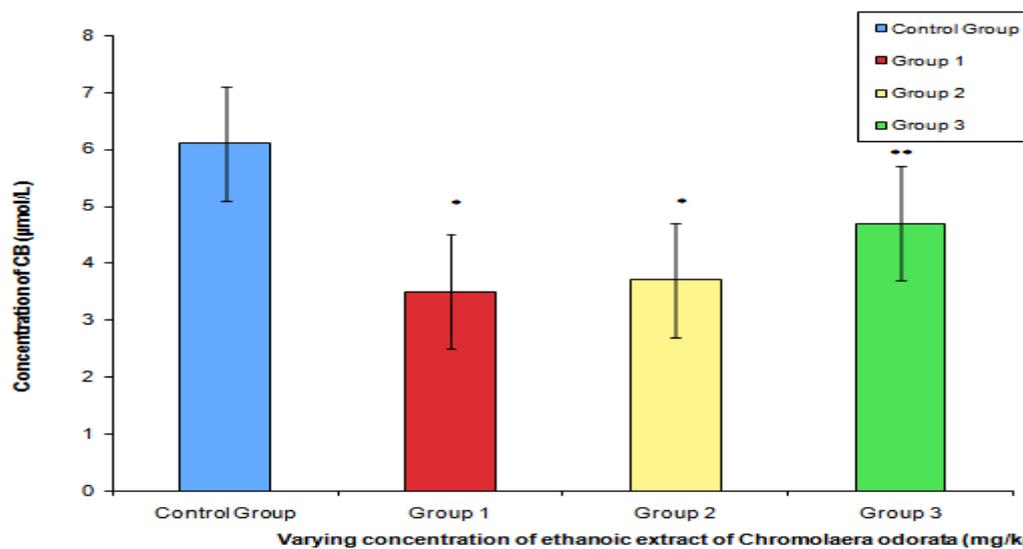


Fig. 3. Effect of different doses of *Chromolaena odorata* extract (100, 150, 250 mg/kg) on conjugated bilirubin (CB) levels of Wister rats (p<0.05)

*significant as to control group

** = significant as to control group, group 1 and 2

Mean ± S.E.M = Mean values ± Standard error of mean.

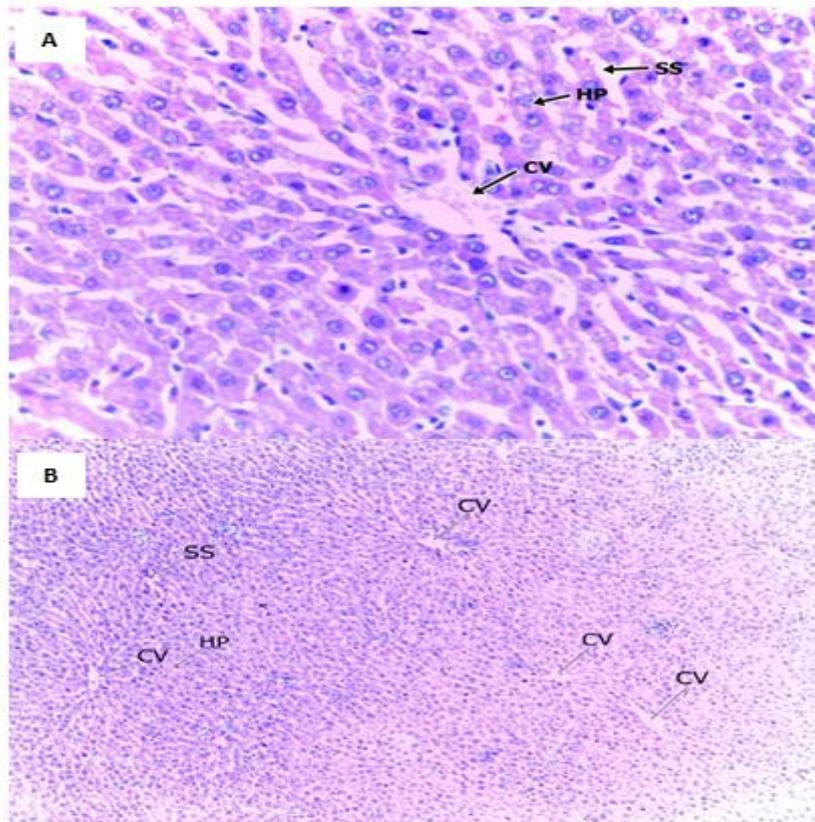


Fig. 4. Control sections of the liver stained with Haematoxylin and eosin technique, showing sinusoidal space (SS), Hepatocytes (HP), central vein (CV). (A) Liver section with magnification of x100. (B) Liver section with magnification of x400.

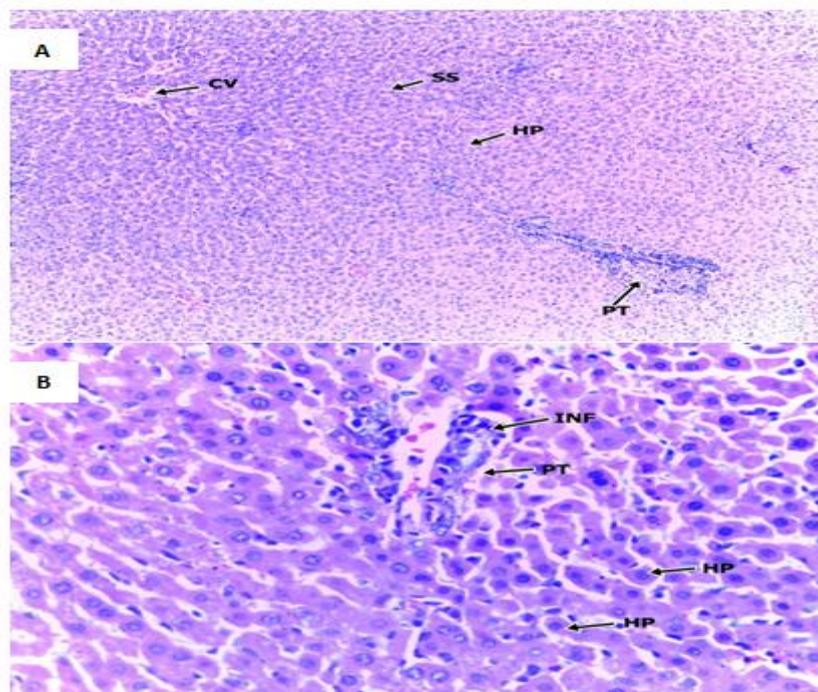


Fig. 5. Sections of liver tissues from group 2 stained with Haematoxylin and eosin technique, showing inflammatory cellular infiltrates (INF), portal tract (PT) and hepatocyte (HP). (A) Liver section with magnification of x100. (B) Liver section with magnification of x400.

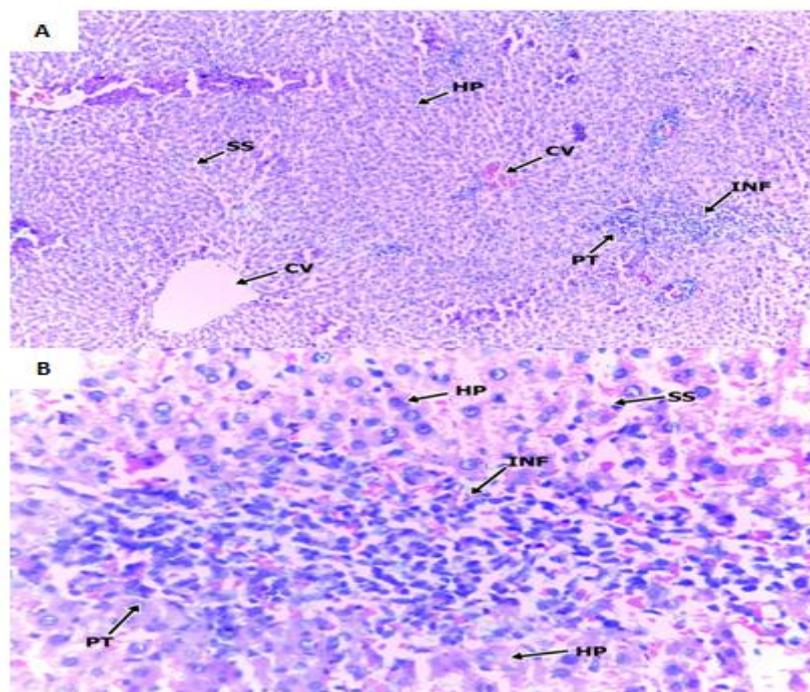


Fig. 6. Sections of liver tissue from group C administered with 250mg/kg of an ethanolic extract of *C. odorata*, stained with Haematoxylin and eosin technique, showing hepatocytes (HP), sinusoidal space (SS), inflammatory cellular infiltrates (INF) and portal tract (PT). (A) Liver section with magnification of x100. (B) Liver section with magnification of x400.

VI. Discussion

The liver is the largest internal organ in a mammalian body. The organ plays many indispensable metabolic functions which include production of proteins, blood clotting factors, glycogen synthesis bile production and detoxification [24]. Many different disease processes can occur in the liver. Some of these conditions include hepatitis, cirrhosis, cancer and other forms of liver injuries due to exposure to chemical substances such as toxins, excessive alcohol, and drugs[25]. *C. odorata* is one the plants that are commonly used as herbal remedies [26, 27, 28].

This study evaluated the toxic effect of ethanolic extract of *C. odorata* on the liver of albino rats. The mean values of TB test groups and control were 5.6, 7.8, 9.4 and 11.1 $\mu\text{mol/L}$ respectively while the men values of CB test groups and control were 3.5, 3.7, 4.7 and 6.1 $\mu\text{mol/L}$ respectively. Results from this study have shown a significant decrease ($P < 0.05$) in the values of total and conjugated bilirubin of the test groups when compared with control. This contradicted the observations made by Nwachukwu *et al.*, [29] and Asomugha *et al.*, [8] who observed a significant increase ($P < 0.05$) in the concentration bilirubin of his test groups when compared with the control. However, there was a continuous increase in the values of total and conjugated bilirubin in the test groups as the concentrations of the extract increased. As a result of this, a significant increase ($P < 0.05$) in the values of conjugated bilirubin among the test groups was observed. This increase in the values of the parameter among the groups may lead to significant increase in the values above the control if the concentrations of the extracts continue to increase as reported by Nwachukwu *et al.*, [29] The presence of pyrrolizidine in *C. odorata* is thought to be the cause of the increase in the concentration of bilirubin due to either excessive red blood cell destruction or inability of the liver to properly eliminate bilirubin. Anyanwu *et al.*, [23] in his previous study had reported increasing values of liver enzymes due to increase in the concentration of the extract, while Asomugha *et al.*, [8] reported a significant increase in the values of liver enzymes due to the effect of exposure to extract of *C. odorata* leaves.

Histological examination of liver tissues after administration of varying concentrations of ethanolic extract of *C. odorata* showed evidence of dosage related hepatotoxicity which is remarkable in test groups 2 and 3 administered with 150 mg/kg and 250 mg/kg of the extract respectively. Sections of liver tissue from group 2 administered with 150 mg/kg of an ethanolic extract of *C. odorata* in figures showed preserved architecture and a central vein that is congested, with prominent hepatocytes radiating outward. The hepatocytes were separated by dilated and congested sinusoidal spaces. The portal areas were dilated, containing marked inflammatory cellular infiltrate with piecemeal necrosis. Sections of liver tissue from group 3 administered with 250 mg/kg of an ethanolic extract of *C. odorata* showed marked portal inflammation and patchy necrosis of hepatocytes. The

sinusoidal spaces are dilated and congested. The central veins are also congested. Some of the hepatocytes have abundant eosinophilic cytoplasm and round to oval nuclei. These features are suggestive of hepatotoxicity in the liver of rats used in this study which is thought to be due to the presence of pyrrolizidine alkaloids in *Chromolaena odorata*. This observation is in agreement with previous studies [17, 30, 31,32]. Pyrrolizidine alkaloids which are also called necine are alkaloids based on the structure of pyrrolizidine. They are produced by plants as a defense mechanism against insect herbivores [33]. This bioactive substance is produced by about 3% flowering plants [34]. Unsaturated pyrrolizidine alkaloids are known to cause cancer and liver damage [35,36].

VII. Conclusion

In the presence of biting economic hardship in some developing countries in Africa and particularly in Nigeria and with non-availability of good health care system, it is obvious that most individuals have resorted to the use of herbal remedies which are readily available and affordable. There should be a regulated use of this medicinal plant since they also may cause adverse effects on the health of the consumers. In this study, the results obtained, have shown that consumption of high dosage of an extract of *C. odorata*, though has been reported to have immense medicinal values, may be dangerous to the liver as has been shown in the albino rats used in this study. Lower dosage of the extract may be safer as previously reported by Okafor *et al.* [17].

Acknowledgement

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Competing Interests

Authors have declared that no competing interests exist.

Authors' Contributions

Anyanwu designed the study, wrote the protocol and the first draft of the manuscript. Eno, Inyang and Asemota managed the analyses of the study. Okpokam performed the statistical analysis. Obioma and Agu managed the literature searches. Emeribe arranged the entire manuscript in its recommended format. All authors read and approved the final manuscript.

Ethical Approval

The Ethical committee for use of animal of the College of Medical Sciences, University of Calabar, was instituted and commenced operation on August, 2017 which was after the period of completion of this present study. However, in order to ensure that the recommended principle for laboratory animal care was duly adhered to in the absence of any examination and approval from the ethical committee, the "Guide the care and use of laboratory animals [37] as used for this study.

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Deficiencies of the work on "Evaluation of Hepatotoxicity Associated with Ethanolic Extract of Chromolaena Odorata in Wistar Rat Model"

- No p values and figures for SEM
- No values or grading for indication of toxicity levels
- No figure to associate liver function parameters (both for TB and CB) with grading for indication of toxicity levels
- No figure to show effect of time of exposure to extract on TB, CB and grading for indication of toxicity levels.
- No figure to show effect of extract's concentration on TB, CB and grading for indication of toxicity levels.
- No figure liver section for group 1 rats

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