Original Article

Effect of ethanolic extract of Chromolaena odorata on the kidneys and intestines of healthy albino rats

Stanley Anyanwu, Imeobong J. Iinyang, Enosakhare A. Asemota, Okechi O. Obioma, Dorothy C. Okpokam, Victoria O. Agu

Department of Medical Laboratory Science, University of Calabar, Calabar, Nigeria
Department of Medical Laboratory Science, Abia State University, Uturu, Nigeria
Department of Pathology, National Orthopaedic Hospital, Kano, Nigeria

Background: The use of plants and plant products for medicinal purposes is an age-long practice in traditional communities and is becoming prominent globally. This study was performed to evaluate the effect of ethanolic extract of Chromolaena odorata on the kidney and intestine of albino rats.

Methods: Twenty growing albino rats with an average weight of 54 g were used in this study. They were grouped into four groups. Groups 1, 2, and 3, known as the test groups, were given 50 mg/kg, 100 mg/kg, and 250 mg/kg ethanolic extract of C. odorata, respectively, while the control group was given distilled water orally. The experiment was performed for 6 weeks. The animals were killed using chloroform suffocation. The kidneys and the intestine were harvested and fixed in 10% neutral buffered formalin for histological analysis. Blood samples were collected from the animals by heart puncture for estimation of creatinine and urea levels.

Results: The creatinine, urea, aspartate aminotransferase, alanine transaminase, and alkaline phosphatase levels of blood sample from the test group were significantly different when compared with the control (p < 0.05). The histological sections of the kidneys in this study showed no signs of degeneration. Infiltration of inflammatory cells and epithelial erosion were observed in the histology sections of the intestine of all the test groups.

Conclusion: The results from this study revealed that uncontrolled use of this plant extract has an adverse effect on the kidney function and on the histology of the intestine of the rats used in this study.

© 2017 Korea Institute of Oriental Medicine. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

The use of plants and plant products for medicinal purposes is an age-long practice in traditional communities and is becoming prominent globally. It has been estimated that 80% of African population use herbal regimen for treatment and control of diseases. This may be because these products are natural and are believed to be potent in addition to rapidly increasing cost of modern health-care services in developing countries including Nigeria. Most plants and plant products are probably safe when normal doses are taken/administered; however, some of them are known to be toxic at high doses, while many others can cause adverse side effects. It has become more worrisome now that Nigeria is passing through economic recession. Most families are resorting to the use of plants and plant products as alternative medicine. *Chromolaena odorata*, which is one of the commonly used plants, is a tropical and subtropical species of flowering shrub in the sunflower family. It is native to North America, mostly seen in Florida and Texas to Mexico and the Caribbean, but it spread to South America, Tropical Asia, West Africa, and parts of Australia. *C. odorata* is known by many names including Siam weed, Christmas bush, devil weed, camphur grass, and common floss flower. In Nigeria, *C. odorata* is commonly known as Ewe Awolowo, Siam weed, Elizabeth weed, Obirakara, Olorohuru, and independent weed (N. Ngozi and T. Osuji “Personal communication on the relevance and indigenous use of medicinal plants”, 2014).

*C. odorata* is a rapidly growing perennial herb, a multi-stemmed shrub growing up to 2.5 m in height 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 vegetation where it can grow up to 10 m in height. The plant is hairy, glandular, and the leaves give off a pungent, aromatic odor when crushed. The leaves are opposite, triangular to elliptical with serrated edges. Leaves measure 4–10 cm (length) × 1–5 cm (width). Leaf petioles are 1–4 cm long. The white to pale pink tubular flowers are in panicles of 10–35 that form at the ends of branches. The seeds are achenes and are somewhat hairy. They are dispersed mostly by wind, but can also cling to fur, clothes, and machinery, enabling long-distance dispersal. Seed production is about 80,000–90,000/plant. Seeds need light to germinate. The plant can regenerate from the roots. In favorable conditions, the plant can grow more than 3 cm/d.

*C. odorata* is used extensively in Nigeria for soil fertility improvement as well as for medicinal and ornamental purposes. The plant is also popularly used for wound healing due to its antimicrobial properties. Concentrations of 0.25 mg/mL and 0.125 mg/mL of ethanolic extract of *C. odorata* exhibited antimicrobial effects against some human pathogens. The anthelmintic properties of the aqeous extracts of *C. odorata* have also been widely known among the rural populations of Africa. Several researchers have reported the wider use of *C. odorata* as an effective therapy against diarrhea, malarial fever, tooth ache, diabetes, skin diseases, dysentery, and colitis. The medicinal values of *C. odorata* are attributed to some specific phenolic compounds that have been isolated from it. However, *C. odorata* also contains a carcinogenic substance called "pyrrolizidine alkaloids." *C. odorata* has been shown to be toxic to cattle and it can also cause allergic reactions. The human kidneys and intestine are important organs of the body. The kidneys play an important role in ultrafiltration and excretion of metabolic waste among other functions. The human intestine digests and absorbs these substances in the system for metabolism. The kidney and the intestine are therefore susceptible to the toxicity from various toxic agents. Chemicals and herbal remedies can induce damages to these organs. In a previous study, the toxic effects of 161.5 mg/kg, 323 mg/kg, 583.5 mg/kg, and 1077 mg/kg of ethanolic extract of *C. odorata* were studied. For the purpose of this study, lower concentrations (50 mg/kg, 100 mg/kg, and 250 mg/kg) will be considered because the higher concentrations already used produced toxic effect on the function of the organs under study. This study is aimed at investigating the effects of ethanolic extract of *C. odorata* on the kidney and intestine of albino rats.

2. Methods

2.1. Plant material

*C. odorata* leaves were collected within the locality of Ikot Offsong Ambai, in Akpabuyo Local Government Area of Cross River State, Nigeria and were identified in the Department of Botany, University of Calabar, Nigeria, with following voucher number MIC(UNICAL)387.

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 ethanolic extract of *C. odorata*.

2.2. Animals

Twenty albino rats of both sexes with an average weight of 56 g were used in this study. Albino rats are species of *Rattus norvegicus* (brown rat). Albino rats have been domesticated for purely scientific experiments and differ from wild rats. Albino rats serve as important animal models for researches in both biomedical sciences and psychology. The rats were bought from the Animal House, College of Medical Sciences, University of Calabar, Calabar, Nigeria. The animals were housed in wire-gauze cages in a well-lit and adequately ventilated room, under standard environmental conditions (12 hours light and 12 hours 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, whereas Group 4 served as the control group. During the experimental
period of 6 weeks, Groups 1, 2, and 3 were given 50 mg/kg, 100 mg/kg, and 250 mg/kg, respectively, of ethanolic extract of C. odorata orally. The control animals (Group 4) were given only distilled water.

2.4. Sample collection and preparation

At the end of the experiments, the rats were anaesthetized with chloroform inhalation and then killed. Blood samples were collected by heart puncture from each of the rats into well-labeled dry plain tubes for biochemical analysis, while the kidneys and the intestine of the animals were harvested and immediately fixed in 10% neutral buffered formalin for histological studies. After 24 hours of fixation, the kidneys and intestine were processed immediately via dehydration of tissue in ascending concentrations of alcohol, cleared in xylene, and infiltrated with paraffin wax before embedding. Sections were cut and mounted on slides and stained with hematoxylin and eosin. The blood samples were collected into already labeled dry plain tubes to determine their urea, creatinine, alkaline phosphatase (AST), and alanine aminotransferase (ALT) levels. The blood samples were allowed to clot. They were 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. The methods used were diacetyl monoxime and modified Jaffé’s methods for urea and creatinine, respectively, while the Armstrong method was used for ALT, AST, and ALP evaluation.

2.5. Statistical analysis

Mean values of urea, creatinine, AST, ALT, and ALP of the test and control groups were compared using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Results were considered significant at p < 0.05.

3. Results

The urea (Fig. 1) and creatinine (Fig. 2) levels in the test groups were significantly raised (p < 0.05) when compared with the control. However, post hoc analysis indicated significantly (p < 0.05) increased level of urea in Group 2, compared with control. It was also significantly (p < 0.05) increased in Group 2, compared with Group 1. Post hoc analysis also indicated significantly (p < 0.05) increased creatinine level in Groups 2 and 3, compared with control. There was a significantly (p < 0.05) increased creatinine level in Group 1, compared with control, and in Groups 2, compared with Group 1. The AST (Fig. 3) and ALT (Fig. 4) levels were significantly reduced (p < 0.05) in the test groups when compared with control. ALP (Fig. 5) levels of the test groups also showed a significant decrease (p < 0.05) when compared with the control. Post hoc analysis of the ALP levels indicated significant reduction (p < 0.05) when the control was compared with Groups 2 and 3 and when the control group was compared with Groups 1 and 3.

3.1. Effects of ethanolic extract of C. odorata on the histology of the kidney

The kidneys showed prominent glomeruli and renal tubules. The glomeruli of the kidneys showed cellular mesangium and distinct Bowman capsule. The renal tubules were closely packed and lined by intact epithelium. The intervening interstitium is scanty. No nephrotoxicity was observed in the kidney sections (Figs. 6 and 7).

3.2. Effects of ethanolic extract of C. odorata on the histology of the intestine

Section of the small intestine in Fig. 8 shows intact layers consisting of the mucosa, submucosa, muscularis externa, and...
the serosa. The mucosa glands are prominent with intact lining epithelium. The covering mucosal epithelium is intact. The submucosal vessels are congested and the glands are prominent.

Section of the intestine in Fig. 9 shows intense inflammatory cellular infiltrates, mainly neutrophils, with erosion of the superficial epithelium. The submucosal vessels are congested, while other layers remain intact.

Section of the intestine in Fig. 10 shows an eroded mucosa with epithelial cell loss and intense submucosal cellular infiltrates. The basement membrane is intact with moderate glandular proliferation.

Section of the intestine in Fig. 11 shows an eroded mucosa with epithelial cell loss and intense submucosal cellular infiltrates. The basement membrane is intact with moderate glandular proliferation.

**Fig. 3** – Comparison between aspartate aminotransferase (AST) levels of the control group and test groups.

*Significant from control group. $p < 0.05$.
† Significant as to Group 1.
‡ Significant from Group 2.
§ Significant from Group 3.

**Fig. 4** – Comparison between alanine aminotransferase (ALT) levels of the control group and test groups.

*Significant from control group. $p < 0.05$.
† Significant as to Group 1.
‡ Significant from Group 2.
§ Significant from Group 3.
Fig. 5 – Comparison between alkaline phosphatase (ALP) levels of the control group and test groups.
*Significant from control group. $p < 0.05$.
† Significant as to Group 1.
‡ Significant from Group 2.
§ Significant from Group 3.

Fig. 6 – Kidney section stained with hematoxylin and eosin. Control group, 400×.
BS, Bowman space; GL, glomerulus; RT, renal tubule.

Fig. 7 – Kidney section stained with hematoxylin and eosin. Group 1, 400×.
BS, Bowman space; GL, glomerulus; RT, renal tubule.
Fig. 8 – Small intestine section stained with hematoxylin and eosin. (A) Control group, 100x; (B) control group, 400x.

G, glands; M, mucosa; ME, muscularis externa; S, serosa; SM, submucosa.

Fig. 9 – Small intestine section stained with hematoxylin and eosin. (A) Group 1, 400x; (B) Group 1, 100x.

G, glands; M, mucosa; ME, muscularis externa; S, serosa; SM, submucosa.

4. Discussion

The intestine accommodates and processes food materials taken into the body. It also absorbs soluble food materials needed for metabolism, allowing the used food materials to be excreted as fecal materials. The kidneys among other functions filter and excrete waste products of metabolism from the mammalian system. Exposure to injurious substances such chemicals and herbal remedies may damage them, resulting in loss of function. The phytochemical constituents of C. odorata, such as allicin, saponins, protease inhibitor, glycosides, flavonoids anthraquinones, terpenes, pyrrolizidine alkaloids, tannins, and sterols are thought to be responsible for its bioactivity.

In this study, it was observed that urea and creatinine levels in the rats used in this experiment were increasing as the concentrations of the extracts increased, which is in agreement with previous work. The significantly increased levels of cre-
atinine and urea observed in all the groups when compared with the control ($p < 0.05$) as observed in this work may be as a result of the adverse effect of the extract on the kidneys. Significantly decreased levels of AST, ALT, and ALP were observed at the concentration of the extract used in this study. This was contrary to the observation made by Asomugha et al., who used higher concentrations of the extract (161.5 mg/kg, 323 mg/kg, 583.5 mg/kg, and 1077 mg/kg). However, in both studies, as the concentrations of the extract increase, the values of the parameters also increased. The increasing enzyme activities observed in this study may be due to increasing concentration of the *C. odorata* extract.

Pyrrolizidine alkaloids are one of the major contents of *C. odorata*. These are a known carcinogen and exhibit hepatotoxicity. They also cause hepatic veno-occlusive disease as well as liver cancer. It is produced by plants as a defense mechanism against insect herbivores. This increase in the levels of creatinine and urea may be due to the toxic effect of pyrrolizidine alkaloids on the kidney. However, other factors such as stress and starvation may have also played
a significant role in the increase due to increased protein metabolism. This observation is in agreement with Asomugha et al’s work. The animals were observed during the last 2 weeks of the experiment to have reduced rate of feeding, which may be attributed to stress. Urea and creatinine could be used to evaluate the functional capacity of the nephron of the kidneys; the significantly raised levels obtained from this work may be an indication of defective functional state. The sections of the kidney, however, did not show any obvious histopathological changes despite significantly increased values seen in urea and creatinine levels observed in the test groups. This result may be an indication of a functional rather than a structural derangement that is usually seen in the acute setting, which may be characterized by abnormal changes in renal functional indices without abnormalities in the histopathological structure. This finding may be followed by structural defects during a long period of exposure to this extract.

Before the administration of the extract, the excreta of all the groups were soft. However, toward the end of the experiment, Group 1 administered with a high dose of the extract (250 mg/kg) produced formed excreta. *C. odorata* has been reported to have antidiarrheal effect with electrolyte reabsorption, which may be the possible reason for the formation of formed excreta in the group administered with the high dose of the ethanolic extract of *C. odorata*. Histological sections of the intestine from all the test groups (1, 2, and 3) showed evidence of inflammatory cells infiltration, which are mainly neutrophils, as well as epithelial erosion. The presence of neutrophils is attributed to acute inflammatory response, which may be due to exposure to extract. Results from this study therefore suggested that the use of extract of *C. odorata*, although had been reported to have nutritional, environmental, as well as medicinal benefits, may have adverse effect on the kidney functions and the histology of the intestine, especially when taken at a higher dosage and for a longer period as evident in the selected kidney functions and the histology of the intestine of the rats used in this study.

Conflicts of interest

The authors have no conflicts of interest to declare.

REFERENCES

1. Hugo WB, Russell AD. Pharmaceutical microbiology. 6th ed. Hoboken, NJ: Blackwell Science Publishers; 2013.

2. Frantisek S. The national guide to medicinal herbs and plants. London, UK: Tiger Books International; 1991:6–20.

3. King RM, Robinson H. Chromolaena odorata (Linnaneus). In: Pharmacology. Vol. 21. New York: Bronx Park; 1970:544–5.

4. Lalith G. Invasive plants: a guide to the identification of the most invasive plants of Sri Lanka. Colombo: Lalith Gunasekera; 2009:116–7.

5. Uyi OO, Ekhator F, Ikuobide CE, Borokini TI, Aigbokhan EL, Egbon IN, et al. Chromolaena odorata invasion in Nigeria: a case for coordinated biological control. Manag Biol Invasion 2014;5:377–93.

6. Odugbemi T. Outlines and pictures of medicinal plants from Nigeria. Lagos, Nigeria: University of Lagos Press; 2006:1–283.

7. Mbaikuja CS, Obeagu EJ, Chude CN, Ibezie OE. Antimicrobial effects of Chromolaena odorata on some human pathogens. Int J Curr Microbiol Appl Sci 2014;3:1006–12.

8. Akinmoladun AC, Akinloye O. Effect of Chromolaena odorata on hypercholesterolemia-related metabolic imbalances. In: Proceedings of the Akure-Humboldt Kellogg/3rd SAAT Annual Conference on Medicinal Plants. 2007:287–90.

9. Metwall AM, Ekejinba EC. Methoxylated flavonoids and flavones from Eupatorium odoratum. Plant Med 1981;42:403–4.

10. Fu FP, Yang YC, Xia Q, Chou MC, Cui YY, Lin G. Pyrrolizidine alkaloids-tumorigenic components in Chinese herbal medicines and dietary supplements. J Food Drug Anal 2002;10, 198–21.

11. Asomugha RN, Okafor PN, Ijieh II, Orisakwe OE, Asomugha AL, Ndefo JC. Toxicological evaluation of aqueous leaf extract of Chromolaena Odorata in male Wistar albino rats. J Appl Pharm Sci 2013;3:89–92.

12. Krinke GJ, History, strains and models. In: Bullock GR, Bunton T, editors. The laboratory rat. London: Academic press; 2000:3–16.

13. Vandenbergh JC. Use of house mice in biomedical research. ILAR J 2000;41:133–5.

14. Asomugha RN, Okafor PN, Ijieh II, Orisakwe OE, Asomugha AL. Hepatic effects of aqueous extract of Chromolaena odorata in male Wistar albino rats. Pharmacologyonline 2014;1:127–36.

15. Randominska-Pandy A. Invited speaker. Drug Metab Rev 2010;42:1–2.

16. Pfaller W, Gstraunthaler G. Nephrotoxicity testing in vitro—what we know and what we need to know. Environ Health Perspect 1998;106(Suppl 2):559–69.

17. Aba P, Joshua P, Ezeonuogu F, Ezeja M, Omojo V, Umeakuana P. Possible anti-diarrheal potential of ethanolic leaf extract of Chromolaena odorata in castor oil-induced rats. J Complement Integr Med 2015;12:301–6.

18. Harsh M. Textbook of Pathology. 6th ed. New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd.; 2010:130.