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Hepatotoxic Effects of Methanol Leaf Extract of *Chromolaena odorata* on Liver Biomarkers and Histology in Wistar Rats

[*1]Adedoyin A. Tologbonse, ,[2]Ngozi A. Onwuka, [3]Hillary E. Otimanam, [1]Armstrong C. Ukah, [1]Mmaduabuchi N. Omeluko, [1]Ejika I. Abam, [4] Peter U. Iseh, [1]Nkechi J. Onyeukwu, [5]Archibong A. Micheal, [1]Susana A. Attah, [1]Titilayo E. Tologbonse, [1]Elochukwu G. Chukwuemelie and [1]Herbert O.C. Mbagwu.

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
[2] Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, Rivers State University, Port Harcourt, Nigeria.
[3] Department of Clinical Pharmacology and Therapeutics, University of Uyo Teaching Hospital, Uyo, Nigeria.
[4] Department of Pharmacology and Therapeutics Bingham University, Nassarawa State, Nigeria.
[5] Department of Medical Laboratory Services (Histopathology), University of Uyo Teaching Hospital, Uyo, Nigeria.

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ABSTRACT

Background: *Chromolaena odorata* plant is widely used in traditional medicine for treating various ailments, including diabetes, malaria, wounds, gastrointestinal disorders and inflammation. This study was designed to determine the effect of *C. odorata* on Liver biomarkers and the histological architecture integrity of the liver in Wistar rat models.

***Methods*:** Matured Wistar rats 180 g – 200 g were divided into four groups of five animals each. The rats were administered with *C. odorata* methanol extracts via oral route for 28 days consecutively and all animals were utilized in the in-*vivo* studies. Standard liver function tests were done using colorimetric method for determination of specific enzymes/biomarkers. Histological analysis was performed on the harvested liver using Mayer's hematoxylin and eosin staining techniques.

Results: The findings showed reduction in the total bilirubin, ALT and AST while the levels of ALP and direct bilirubin increased. This is suggestive of a potential hepatoprotective effect of the extract at moderate doses and hepatic dysfunction or membrane leakages at a high dose (821.58 mg/Kg), these results were significant at $p \leq 0.05–0.01$ when compared with the control. Histological findings suggest that low doses were safe.

***Conclusion*:** The extract exhibited dose-dependent hepatotoxic effects on liver enzymes. Low doses are safe, while higher doses induced a moderate altered hepato-architecture with areas of degenerated hepatic and ductal cells, indicating a need for careful dose optimization. These findings validate the traditional use of *C. odorata* in treatmet of gastrointestinal disorders and suggest its potential as a natural therapeutic agent.

Keywords: *Chromolaena odorata*, hepatotoxicity, histopathology, liver biomarkers, methanol extract

.1. INTRODUCTION

Chromolaena odorata (Asteraceae) and *Chromolaena odorata (L)* King, commonly known as Siam weed, is a fast-growing perennial and invasive weed native to South and Central America. It has been introduced into the tropical regions of Asia, Africa and other parts of the world. *C. odorata* is also known by various other names such as Armstrong's Weed, Baby Tea, Jack in the bush, King Weed, Paraffin Bush, Paraffin Weed Bitter Bush, Butterfly Weed, Christmas Bush, Devil Weed, Eupatorium, Turpentine Weed and Triffid Weed [1]. *C.odorata* L. has a short life cycle of approximately ten years. It occasionally reaches its maximum height of 6m as climber on other vegetation. The root formation is fibrous and flowers are white or pale bluish-lilac [2]. It is an aggressive competitor that occupies different types of lands where it forms dense strands that prevent the establishment of

Corresponding author: Email: adedayotologbonse@uniuyo.edu.ng; **Phone: +2348037801881**

other flora. It is a menace in plantations and other ecosystems. It suppresses young plantations, agricultural crops and smothers vegetation as it possesses allelopathic potentialities and growth inhibitors [3-5]. The economic value of *C. odorata* is low. Consequently, there is relative paucity of research works on it. In recent decades, it has become a serious weed in the humid tropics of Southeast Asia, Africa and Pacific Islands. Following its introduction to Nigeria, the weed quickly spread through eastern Nigeria in the 1940s and was first reported by Ivens in 1974 to the west of the River Niger in 1955 and from Lagos and its environs in 1960, from where it might have spread into Benin Republic and other West African countries. By 1960, *C. odorata* had occupied the south-eastern states of Nigeria, and possibly spread from there into Cameroon ([6-8]). It spreads rapidly in lands used for forestry, pasture and plantation crops such as rubber, coffee, coconut, cocoa and cashew. The plant is poisonous to livestock as it has exceptionally high level of nitrate (5 to 6 times above the toxic level) in the eaves and young shoots; it can result in tissue anoxia when fed to cattle [9]. Despite the negative effects of the plant, it has patronage from practitioners of traditional medicine.

Figure 1. Leaves and flowers of *Chromolaena odorata* L - Source: Field Source [10].

In the southern part of Nigeria, the leaves are used for wound dressing, skin infection and for haemostasis. The fresh leaves of *C. odorata* or the decoction have been used by practitioners of traditional medicine for the treatment of human burns, soft tissue wounds, ulcerated wounds, burn wounds, postnatal wounds and for the treatment of leech bites, indigestion and skin infection. It is also used for the treatment of various ailments, such as amenorrhea, catarrh, cold-associated nasal congestion, diabetes, diarrhoea, fever, pertussis and rheumatism, and as a vermifuge [11]. Other pharmacological properties of this plant include anthelminthic [12], antimalarial, analgesic [13, 14], anti-inflammatory, antipyretic, antispasmodic ([15-17]), antimycobacterial, insecticidal, antioxidant, anti-gonorrheal), fungicidal, diuretic [18, 19], blood coagulating, and antimicrobial effects [20]. The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. Despite the widespread use of *C. odorata*, its impact on liver remains poorly understood. While some studies explore the plant's anti-ulcer properties, a focused examination of its effect on the liver integrity is currently lacking. Given the increasing use of traditional medicine in the health sector, research is needed to determine whether *C. odorata* extracts have any direct effects on the liver, a major metabolic organ; especially as extract of *C.odorata* is often taken via the oral route when used as herbal mixture for treatments. Phenols have been reported as one of the essential constituents in *C. odorata*. The structure contains a hydroxyl group, a property that is responsible for the scavenging effect of this plant [21]. This study aimed at evaluating the hepatotoxic effect of the methanol extract *C. odorata* on the biomarkers and the histology of the liver in Wistar rats' model.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological materials

Fresh leaves of *Chromolaena odorata* were collected from Ekebedi Oboro, Ikwuano LGA, Abia State, Nigeria. The plant was identified and authenticated by a Taxonomist of the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria, and deposited in the Faculty of Pharmacy Herbarium with a specimen No. UUPH No.10 (c). Twenty (20) Wistar rats were obtained from University of Uyo animal house and they were allowed to acclimatize in the laboratory for a period of seven days. They were allowed free access to feed and water *ad libitum* throughout the period of the experiment. The rats were kept in wooden cages furnished with hardwood chip bedding with ambient temperatures of 28° Celsius.

2.1.2 Chemicals and reagents

Distilled water, 10% buffered formalin, ketamine, Atropine, tween 80 (polysorbate 80), 10% formalin and other chemicals. All drugs and chemicals were of standard analytical grades and were purchased from the Pharmacy store of the University Uyo Teaching Hospital, Uyo and a few other chemicals gotten from the Laboratory of the Department of Pharmacology and Toxicology, University of Uyo, Uyo, Nigeria.

2.1.3 Equipment

Automated analyzers and Fortress Diagnostic Kits (Fortress Diagnostic Limited, UK), rotary evaporator, electronic microscope, Aluminum foil, cotton wool, syringes (5ml), cannula, masking tapes, disposable hand gloves and nose mask, breakable plates, beakers (250ml,1000ml), surgical scissors, organ bottles, plain bottles, Electronic weighing balance, triple beam balance, stirring rods, pot, Whatman No. 1 filter paper.

2.2 Methods

2.2.1 Preparation of Drugs and extraction

Fresh leaves of *Chromolaena odorata* were carefully separated from the stalks, washed free of sand and debris under a running tap water and rinsed with distilled water. The fresh leaves were shade-dried for a period of two weeks. The dried leaves were chopped into pieces and progressively turned into powder, with the use of an electric grinding machine. The resulting powder (400g) was macerated exhaustively in 1500 ml of 80% methanol for 72 hours and filtered through a filter paper (Whatman no.1) to obtain a methanolic crude extract, the percent yield was 28.1%. The extract was concentrated to dryness in a rotary evaporator at 40ºC to yield a dried extract of *Chromolaena odorata,* all treatment were carried out via the oral route with the aid of oropharyngeal cannula has indicated below:

$$\text{Volume administered (mL)} = \frac{\text{weight of the animal (kg) x required dose (mg/kg)}}{\text{Concentration of test drug (mg/mL)}}$$

2.2.2 Study Design for in vivo procedures/extract treatments

Twenty (20) Albino rats weighing between 180 g to 200 g were randomly selected from the animal house unit, Department of Pharmacology and Toxicology and the rats were subdivided into four (4) groups (with n=5 per group) of control, *C .odorata* extract (273.86 mg/Kg), *C. odorata* extract (547.72 mg/Kg), and *C. odorata* extract (821.58 mg/Kg). The rats were divided into four (4) groups (n=5). Group 1, rats were administered distilled water (5ml/kg) only as negative control; group 2 were also administered *C. odorata* extract (273.86 mg/kg) body weight; group 3 were administered *C. odorata* extract (547.72 mg/kg); group 4 were administered *C.odorata* extract (821.58 mg/kg) based on the LD_{50} extrapolation of one- third doses from the previous study. The period of treatment was twenty-eight (28) days. At the end of the 28 days of treatment, the animals were fasted for 24 hours after which, they were euthanized with 50 mg/Kg of ketamine as an anesthetic agent then on paralysis, they were sacrificed and the liver harvested and further dissected into two parts.

2.2.3 Experimental Procedure in biochemical Analysis for Liver Function parameters

Liver functions were assessed by evaluating biochemical parameters, including:

Alanine aminotransferase (ALT): Measured using the Reitman-Frankel colorimetric method for *in vitro* determination of GPT/ALT in serum or plasma [22]; with the use of Quimica Clinica Applicada (QCA) test kits (Spain). Aspartate aminotransferase (AST): Measured by the Reitman-Frankel colorimetric method for *in vitro* analysis of GOT/AST in serum or plasma [22]. *Alkaline phosphatase (ALP):* Determined using the phenolphthalein monophosphate method for *in vitro* analysis in serum or plasma [23]. *Total and direct bilirubin*: Levels were determined using the colorimetric diazo method as described by [24], serving as markers of hepatic function. In this study, all the biochemical parameters estimated were carried out using automated analyzers and Fortress Diagnostic Kits (Fortress Diagnostic Limited, UK), according to standard procedures of the manufacturer's protocol at the University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria.

2.2.4 Liver Sample Collection, Histological Processing and Histopathological Examination/Evaluation

The histological assessment of liver tissues following treatment with varying doses of *Chromolaena odorata* leaf methanolic extract was carried out as described below according to the method as described [25]. In the Wistar rat model used, all the animals were weighed and fasted 24 hours after the last treatments with the appropriate doses of the *C. odorata* extract Blood samples were collected by cardiac puncture The liver tissues were aseptically excised and the first part that was fixed in 10 % buffered formalin and sent for histopathological analysis studies. The second part washed with cold physiological saline solution to remove blood stains and then stored in ice cold normal saline for biochemical analysis. The liver samples that was fixed in 10% buffered

formalin were processed using standard histological techniques. The tissues were embedded in paraffin wax, sectioned into thin slices (typically 4–5 µm), and stained with Hematoxylin and Eosin (H&E). This staining enabled the microscopic evaluation of hepatic architecture, cellular integrity, and pathological alterations such as necrosis, degeneration, inflammation, vascular congestion, and sinusoidal dilation. Prepared liver sections were examined microscopically to evaluate histological features, including hepatic cord (liver plate) arrangement, central vein structure, and sinusoidal integrity. Specific pathological features assessed included Kupffer cell proliferation, fatty degeneration, hepatocellular necrosis, and sinusoidal congestion, following the criteria outlined by [25]. Standard experimental protocols were observed, Faculty of Pharmacy, University of Uyo, ethical committee's clearance was obtained, in line with the Principle of Laboratory Animal care [26].

2.3 Statistical Analysis

Results were presented as mean ± standard error of mean (SEM). Analysis of data was done using SPSS software package employing the one-way ANOVA followed by Dunnett post Hoc test with significant differences at $P<0.05$ and $P<0.01$ respectively.

3. RESULTS

3.1 Effects of the Leaf Extracts on ALT, ALP, AST, TB, and DB Levels in Experimented Wistar Rats

The methanol leaf extracts of *Chromolaena odorata* significantly increased the levels of alkaline phosphatase (ALP) and direct bilirubin (DB) levels; while significantly decreasing alanine transferase (ALT), aspartate transaminase (AST), and total bilirubin (TB), in the groups of treated Wistar rats. The effects were more pronounced at the highest dose (821.58 mg/kg). These changes were statistically significant at ($p \leq 0.05$–0.01) when compared with the control.

Table 1: Liver function test-Effect of methanol leaf extract of *Chromolaena odorata* on ALT, ALP, AST, TB, and DB Level in Experimental Wistar Rats

Group/ x̄	ALT(U/L)	ALP(U/L)	AST (U/L)	TB (mg/dL)	DB (mg/dL)
Control	19.68±0.90	19.90±0.55	82.82±5.25	16.30±0.55	1.20±0.03
273.86mg/kg	17.80±0.85*	23.40±0.95	57.00±4.50*	14.96±0.60	2.50±0.12*
547.72mg/kg	19.20±1.05	24.00 ±1.00*	38.40±3.10*	15.08±0.77*	1.80±0.05
821.58mg/kg	16.10±1.25*	23.60±1.15*	10.40±0.45*	12.36±0.69*	3.12±0.23*

x̄ = Mean ± SEM, n = 5; * = Significant = $p \leq 0.05$

3.2: Liver Histological Results

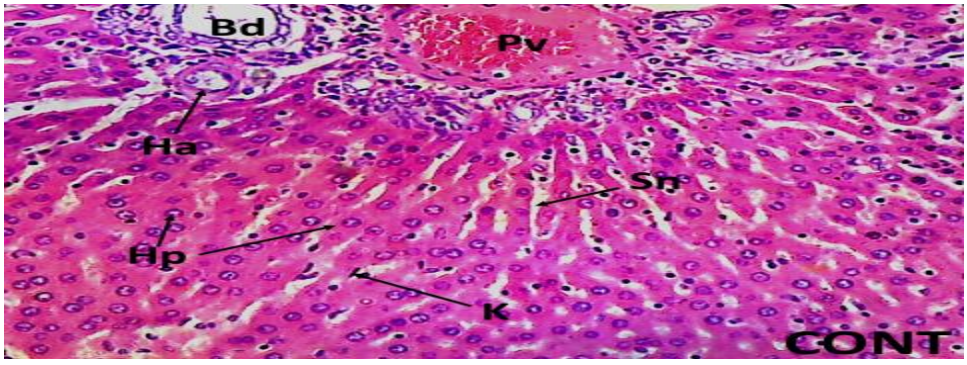

Figure 2: Photomicrograph of a section of a control liver tissue showing normal hepatic architecture with well protected portal vein (Pv), hepatic artery (Ha) and Bile duct (Bd), within the portal area, well-protected hepatocytes (Hp), presence of kupffer cells (K), and radiating sinusoids (Sn) within the hepatic lobules (H&E x100). Inference: Not affected.

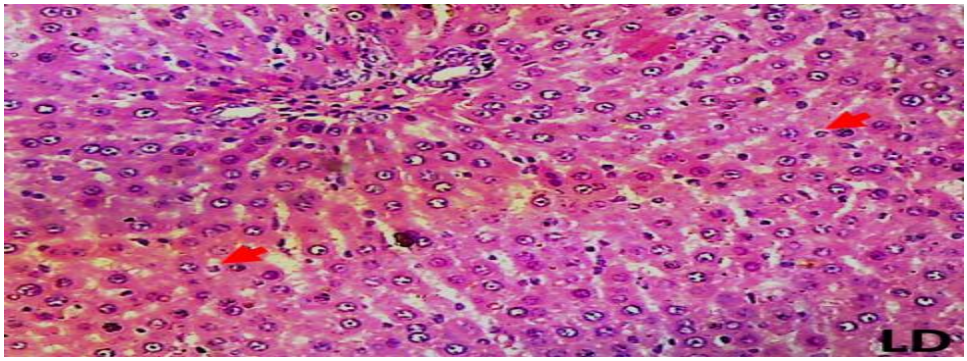

Figure 3: Photomicrograph of a transverse section of a treated liver tissue of Group 1 (Low Dose- 273.86 mg/Kg); with demonstrate a mildly altered hepato-architecture having space areas of degenerating hepatic cells with vacoulations (red arrow) within the hepatic lobules. (H&E x100). Inference: Mildly affected.

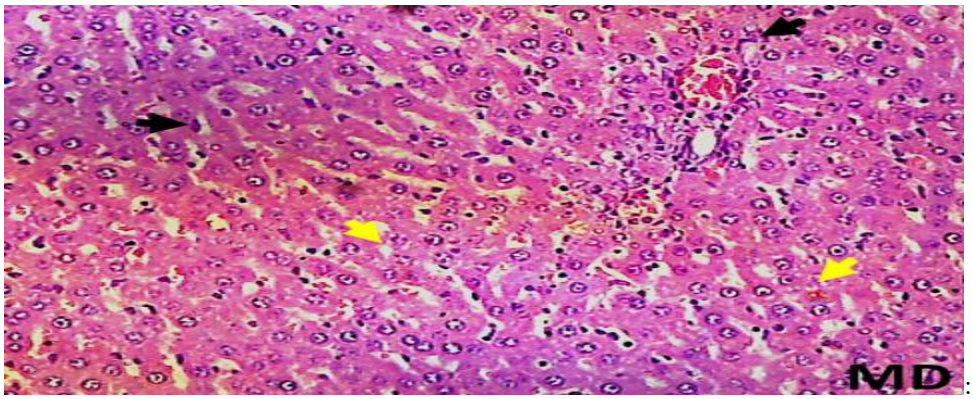

Figure 4: Photomicrograph of Group 2 (Medium Dose- treated with 547.72 mg/Kg); mildly affected treated liver tissue demonstrating a moderately altered hepato-architecture with areas of degenerating hepatic cells (black arrow), spreading micro-vesicular steatosis (yellow arrow), and increase proliferation of kupffer cell within the hepatic lobules. (H&E x100). Inference: Moderately affected.

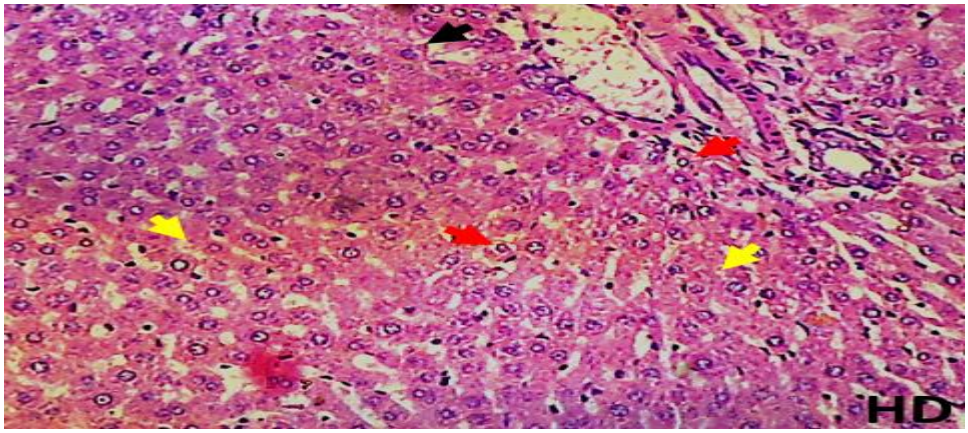

Figure 5: Photomicrograph of a transverse section of a Group 3 (High Dose- 821.58 mg/Kg) treated liver tissue demonstrating a moderately altered hepato-architecture with areas of degenerated hepatic and ductal cells (black arrow), degenerating and vacuolated hepatocytes (red arrow), and spreading micro-vesicular steatosis (yellow arrow), within the hepatic lobules. (H&E x100). Inference: Moderately affected

4. DISCUSSION

Liver function results showed a statistically significant reduction changes ($p < 0.05$) in aspartate aminotransferase (AST), fluctuating alanine aminotransferase (ALT) levels with reduction at higher doses, and a significant increase in alkaline phosphatase (ALP). Total bilirubin decreased at higher doses, whereas direct bilirubin increased, suggesting possible alterations in hepatic metabolism and biliary function. ALP is an important enzyme associated with the hepatobiliary system and is often elevated in conditions involving bile duct obstruction or cholestasis [27]. Alanine aminotransferase (ALT) is primarily localized within hepatocytes; they are mainly released into the bloodstream when liver cell membranes are damaged [28]. The reduction in the total bilirubin alongside decreased

AST and ALT activities, suggests a potential hepatotoxic effects of the extract at high doses. Elevated direct bilirubin and alkaline phosphatases in all the treated groups are indicative of hepatic dysfunction or membrane leakage. Low doses of the extract of *C. odorata* seem to exhibit profound extract-mediated suppression points to stabilization of hepatocellular membranes and reduced oxidative damage, hence hepatoprotective. Phytochemicals in *C. odorata*, particularly flavonoids and tannins, possess antioxidant and membrane-stabilizing properties, which could underline these protective effects [29-31]. *C. odorata* at a moderate dose of (547.72 mg/kg) seems to have similar effects with Azithromycin; as previous study had reported that Azithromycin at 30mg/kg when administered to adult rats for fifteen days, does not affect the body weight gain or relative liver weight in treated rats; whereas it significantly ($p<0.01$) decreased the levels of alanine aminotransferase(ALT), alkaline phosphatase(ALP) and aspartate aminotransferase(AST), superoxide dismutase(SOD) and catalase activities in the liver [25]. Another previous study revealed that the LD_{50} of *C.odorata* is 2738.61 mg/kg, indicating that *C. odorata* methanolic extract has low acute toxicity, as it is well above the threshold for substances considered highly toxic (<50 mg/kg) [32]. These findings support the traditional use of *C. odorata* in herbal medicine, as it suggests a wide safe therapeutic window at low to moderate doses. These findings aligned with previous studies reporting antispasmodic properties of *C. odorata* [31], supporting its potential use in treating gastrointestinal disorders characterized by spasms, such as irritable bowel syndrome; this is often related to the liver, a major accessory organ of the digestive system. The histological findings provide critical insights into the safety and potential toxicity of *C. odorata* extract on liver architecture. The control group (treated with distilled water) and the low-dose group (273.86 mg/kg) exhibited normal histological features, (Figures 3). These findings indicate that low doses of *C. odorata* extract are safe and do not induce structural damage to the liver architecture. In contrast, the medium-dose (547.72 mg/kg) and high-dose (821.58 mg/kg) group showed moderate damage, (Figures 4 and 5). These changes suggest that higher doses of the extract may induce moderate liver damage, hence showing moderately altered hepato-architecture with areas of degenerated hepatic and ductal cells, degenerating and vacuolated hepatocytes, and spreading micro-vesicular steatosis, within the hepatic lobules. potentially due to cytotoxic compounds such as alkaloids or high nitrate levels [9]. However, the absence of severe or necrosis indicates that the damage is moderate and potentially reversible. These findings correlate with the acute toxicity results of a previous study, where higher doses of *C. odorata* (3000 mg/kg and above) was reported to have caused significant toxicity [33]. The moderate effects observed at 547.72 mg/kg and 821.58 mg/kg suggest a dose-dependent toxic effect on the liver, which may limit the therapeutic use of *C. odorata* at higher doses. The presence of bioactive compounds, such as phenolic acids and flavonoids, may contribute to both the therapeutic and toxic effects, as these compounds can have dual roles depending on concentration applied [34].

5. CONCLUSION

The findings from this study suggest that a higher doses of *C. odorata* exhibit a moderately altered hepato-architecture with areas of degenerated hepatic and ductal cells; and spreading micro-vesicular steatosis (indicating hepatotoxic effects/ liver injury); while low doses exhibit mild effect, which may suggests a potential hepatoprotective effect of the extract at moderate doses and may also give support to the safety profile of *C. odorata* for traditional medicinal uses, especially at low to moderate doses: indicating a need for careful dose optimization.

DECLARATIONS

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Conflicts of interest

The Authors express no conflicts of interest in this research work among the team.

Authors contributions

Authors 4,5 and 6 are the primary investigators, while authors 2 and 3 are co-investigators, authors 1 and 13 are the main supervisor and co-supervisor respectively; authors 9,10 and 11 provided the needed technical assistance, finally authors 7, 8 and 12 assisted in data analysis and interpretation of results.

Ethical approvals

Ethical Approval Ethical standard and procedures were observed; The Faculty of Pharmacy, University of Uyo ethical committee's clearance was obtained, in line with the Principle of Laboratory Animal care [26].

6. REFERENCES

[1]. Fadyen MC, 2004. *Chromolaena odorata (L)* King and Robinson (Asteraceae), commonl names

[2]. Cruttwell RE. The ecology and control of *Chromolaena odorata*. In R. Muniappan (Ed.), *Proceedings of the Third International Workshop on Biological Control and Management of Chromolaena odorata*. Agricultural Experiment Station, University of Guamweed, 1996; 1–9.

[3].Ambika SR, Jayachandra. Allelopathic potential of *Chromolaena odorata* (L.) R.M. King and H. Rob. *Current Science,*1980*;49*(23), 886–888.

[4]. Ambika SR, Jayachandra. Allelopathic effects of *Chromolaena odorata* on subsequent crops. *Indian Journal of Weed Science* 1982; *14*(2), 94–98.

[5]. Muniappan and Marutani, 1988. *Chromolaena odorata and* its allelopathic potentialities and growth inhibitors Properties. [6].Ivens GW. The problem of *Chromolaena odorata* in Nigeria. *PANS Pest Articles and News Summaries,* 1974; *20*(2), 140–143.

[7]. Odukwe DO. *Chromolaena odorata* in Nigeria. *Nigerian Agricultural Journal,*1965; *2*(1), 10–12.

[8]. Hoevers, M'boob. The spread of *Chromolaena odorata* in eastern Nigeria and the Africa subregions;. *Weed Science Bulletin,*1996; *5,25.*

[9]. Sajise PE, Palad MB,Lales PN. Nitrate toxicity in livestock due to *Chromolaena odorata*. *Philippine Weed Science Bulletin,* 1974; *1*(1), 12–16.

[10]. Tologbonse AA, Onwuka NA, Otimanam HE, Amstrong I O, Omeluko NM, Ejike, IA. Field Source, Uyo: Leave and flowers of *Chromolaena odorata* L .*West African Journal of Pharmacy*, 2025;36(2);1-12.

[11]. Goodall JM, Erasmus DJ.1996. Review of the status and integrated control of the invasive alien weed, *Chromolaena odorata*, in South Africa. *Agriculture, Ecosystems and Environment, 56*(3), 151–164.

[12] Patel JJ, Acharya SR , Acharya NS. Anthelmintic activity of *Chromolaena odorata* (L.) King and Robinson. *International Journal of Pharma Sciences and Research,* 2010; *1*(3), 171–174.

[13]. Ongkana .2003. Antimalarial, Other pharmacological properties of *Chromolaena odorata* (L.)

[14]. Chakraborty AK , Rambhade S, Patil UK. *Chromolaena odorata* (L.): An overview. *Journal of Pharmacy Research,* 2011; *4*(3), 573–576.

[15]. Oludare BE, Tijani AA, Oyeniran OI . Pharmacological properties of *Chromolaena odorata*. *African Jo,urnal of Medicine and Medical Sciences,* 2000; *29*(3–4), 233–237.

[16]. Phan TT, Hughes MA, Cherry GW. Effects of *Chromolaena odorata* extract on cultured human keratinocytes and fibroblasts. *Journal of Wound Care,* 2001; *10*(1), 504–510.

[17]. Caceres A, Menendez H, Mendez E, Cohobon E, Samayoa BE, Jauregui E, Peralta E, Carrillo G . Antigonorrhoeal activity of plants used in Guatemala for the treatment of sexually transmitted diseases. *Journal of Ethnopharmacology,* 1995; *48*(2), 85–88.

[18]. Gopinath SM, Suneetha TB, Naik DR. Diuretic activity of *Chromolaena odorata* (L.) R.M. King and H. Robinson. *Pharmacologyonline,* 2009; *3*, 345–350.

[19] Triaratana T, Suwannawong S, Na-Bangchang K. Blood coagulating effects of *Chromolaena odorata*. *Journal of the Medical Association of Thailand,* 1991; *74*(4), 216–220.

[20]. Borges *et al.,* 2016. Traditional uses: blood coagulating, and antimicrobial effects of *Chromolaena odorata leaves.*

[21]. Pisutthanan N, Liawruangrath S, Bremner JB. Chemical constituents of *Chromolaena odorata*. *Natural Product Research,* 2006; *20*(7), 636–640.

[22]. Reitman S, Frankel S. A. Colorimetric method for Determination of Serum glutamic Oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical pathology,* 1957; 28:56-62.

[23]. Klein B, Read PA, Babson AL.Rapid method for the qualitative determination of serum alkaline phosphatase. *Clinical Chem*., 1960;6:269-275.

[24]. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter method.*Journal of Biol Chem*., 1937;119:481-490.

[25]. Paulose P, Juliet S, Samraj S, Nair SN, Chandrasekhar L, George AJ, Narayanan G, John K, and Rudolph R. Ameliorative effects of vitamin E on azithromycin induced biochemical and Histological changes in the liver tissues of rats.*Pdf Academia.edu, Advanced Animal Vet.Sci* 4(2), 85-91,2016.

[26]. NIH 1996. Principle of Laboratory animal care of the National Institute of Health –NIH Publication No. 85-123) guidelines.

[27]. Burtis CA, Bruns DE. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 7th ed. Indian Journal of Clinical Biochemistry,2015;30(2)

[28]. Guyton, AC,Hall, JE. Textbook of Medical Physiology ;4th ed. Elsevier.2021.

[29]. Okunade AL. Ageratum conyzoides L. (Asteraceae). Fitoterapia, 2002;73(1);1-16.

[30]. Akinmoladun FO, Komolafe TR, Faromb EO, Oyedapo OO. Evaluation of antioxidants and membrane stabilizing potentials of Chromolaena odorata leaf extract. *Journal of Medicinal Plants Research,* 2010;4(15);1501-1506.

[31]. Eweka AO, Om'Iniabohs FAE. Histological studies of the effects of oral administration of Chromolaena odorata extract on the liverin adult Wistar rats. *International Journal of Morphology,* 2011;29(2);543-548.

[32]. Tologbonse AA, Onwuka AN, OtimanamHE, Ukah CA, Omeluko NM, Abam EI, Ettang UN, Michael AA, Mbagwu HOC, Olorunsola,EE. Evaluation of antispasmodic and toxicity potential of methanol extract of *Chromolaena odorata* on ileum muscle. *West African Journal of Pharmacy*, 2025;36(2);1-12.

[33]. Oludare BE, Tijani AA, and Oyeniran OI. Pharmacological properties of *Chromolaena odorata*. *African Journal of Medicine and Medical Sciences,* 2000; *29*(3–4), 233–237.

[34]. Kouamé PB, Jacques C, Bed G, Silvestre V, Loquet D, Barillé-Nion S. Isolation and characterization of three new compounds from *Chromolaena odorata* with cytotoxic and anticancer properties. *Phytochemistry Letters,* 2013; *6*(4), 559–563.