Tropical Journal of Applied Natural Sciences

Vol. 3 Issue 1 (2025)

Tropical Journal of Applied Natural Science

ISSN: 2449-2043

https://tjansonline.org/view-paper.php?id=72; **Volume/Issue:** Volume 3, Issue 1

Published: July 19, 2025

Effect of Aqueous Extract of *Chromolaena odorata* Leaves on Liver and Kidney Function of Carbon Tetrachloride Induced Hepatotoxicity in Male Wistar Rats

Okafor, Lilian Adaora[1]; Nwaka, Andrew Chibuzo[1]; Okolue, Chinwendu[1]; Nwaka, Chinyere Stella[1]; Obiefuna, Grace Chigozie[2].

[1]Department of Biochemistry Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State.

[2]Integrated Science Department FCE (T.), Umunze.

Address of correspondence: adaoralilian98@gmail.com; +2348066749098.

ABSTRACT

This study investigated the effect of aqueous extract of *Chromolaena odorata* leaves on liver function and kidney function of carbon tetrachloride (CCl_4)-induced hepatotoxicity in male Wistar rats. A total of thirty-eight rats were used in the study. Of these, 13 rats were designated for the determination of the lethal dose (LD_{50}) while the remaining 25 rats were used for the other aspects of the study. The rats were divided into five groups: Group A (normal control), Group B (negative control: induced with CCl_4 and left untreated), Group C (positive control: induced with CCl_4 and treated with silymarin), Group D (CCl_4-induced and treated with 100 mg/kg *C. odorata* extract), and Group E (CCl_4-induced and treated with 300 mg/kg *C. odorata* extract). The LD_{50} was done using Lorke's method while the liver function and kidney function analyses were done using standard biochemical methods. LD_{50} testing indicated no signs of toxicity at both low and high doses of the extract. Liver function and kidney function levels showed improvement with the extract treatment, though Group A (normal control) exhibited superior results compared to Group C (positive control). Overall, the findings suggest that the aqueous extract of *Chromolaena odorata* leaves has a hepatoprotective and antioxidant potential, which may provide a viable therapeutic approach for CCl_4-induced hepatotoxicity.

Keywords: *Chromolaena odorata,* Carbon tetrachloride, Hepatotoxicity, Extract, Liver, Kidney**.**

INTRODUCTION

Among the biggest organs in the human body, the liver is mostly used for extensive metabolism and excretion. Maintaining, controlling, and guaranteeing homeostasis within the body depends much on it (Rajib *et al*., 2009). Acting as a vital biochemical center, the liver is fundamental for metabolism, digestion, detoxification, and bodily drug elimination (Robert, 2008). It also generates proteins and antibodies that help blood clots to form. Important for appropriate physiological activities, the liver tissues control a wide spectrum of metabolic reactions including creation and breakdown of simple and complex substances. The liver is the body's chemical powerhouse; it has no replacement given it’s around 500 vital roles. Mammals cannot survive long with a seriously damaged liver, no question about it.

Continuous exposure to hazardous compounds may cause long-lasting damage to the liver via cell destruction and loss of functional units, therefore possibly compromising its capacity. One of the ways poisons enter the body is via the skin, either purposely or accidently. Hepatotoxicity, or inflammation of liver cells, results from alcohol levels in the blood exceeding the capability of the liver for detoxification. Liver damage may also be produced by viruses or hazardous chemicals formed from the bio activation of drug metabolites (Drake *et al*., 2015). Carbon tetrachloride is a well-known chemical agent used in research to produce liver damage, and its toxic effects have been thoroughly researched. This hepatotoxic chemical leads to liver damage characterized by enzyme leakage into the circulation and centrilobular necrosis. In the past, carbon tetrachloride was employed as a degreaser and cleaning agent in residences, enterprises, textile dry-cleaning laundries, and fire extinguishers. It was also a precursor in the manufacturing of refrigerants and propellants. However, owing to its extreme toxicity, most of its uses are currently prohibited (Teschke, 2018). High exposure levels of carbon tetrachloride may significantly damage the central nervous system and result in liver and kidney deterioration, with chronic exposure providing lethal dangers.

Standard pharmacological therapies for liver problems are sometimes inadequate and may lead to significant side responses. The high cost and restricted availability of conventional medicines, along with the danger of substantial

adverse effects, have driven an increased dependence on medicinal plants for addressing liver-related diseases in developing nations. Numerous plants are now being studied for their medicinal qualities, among which *Chromolaena odorata* stands out.

Chromolaena odorata is a tropical flowering shrub belonging to the sunflower family, Asteraceae. It is endemic to North America, stretching from Florida and Texas to Mexico and the Caribbean, and has been imported to tropical Asia, West Africa, and some regions of Australia. It is frequently known to as Siam Weed, Christmas Bush, Devil Weed, and Common Floss Flower (Lalith, 2009). *Chromolaena odorata* is a quickly growing perennial plant, well renowned for its wound-healing capabilities. Traditionally, it has been employed for different therapeutic uses, notably in healing wounds, skin infections, and inflammation. Research has showed that its leaf extract exhibits antioxidant, anti-inflammatory, analgesic, antibacterial, and cytoprotective activities, among other major medical effects (Suksamrarn *et al.,* 2004). This research intends to explore the influence of aqueous leaf extracts of *Chromolaena odorata* after the development of carbon tetrachloride-induced liver injury in Wistar rats.

MATERIALS AND METHODS

Samples Used: Fresh leaves of *Chromolaena odorata* were purchased from the local market, Eke Awka in Awka South LGA, Anambra State, Nigeria and were authenticated by a Taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, with herbarium number, NAUH-73^{D}.

Reagents and Equipment Used: Sulfuric acid, nitric acid, ethanol, sodium hydroxide (NaOH), sodium hydrogen carbonate ($NaHCO_3$), potassium ferricyanide, sodium nitrite, alkaline solution, distilled water, picric acid, desiccator, incubator, automated pipette, chemical balance, a visible light spectrophotometer with an optically matched set of cuvettes, stop watch.

Sample Processing: The leaves of *Chromolaena odorata* were removed from the stem, washed, sun-dried and then placed in a hot-air oven with temperature controlled at 60^0C. The leaves were ground to powder using electric blender and stored in air tight containers for the process of extraction.

Extraction: Three hundred (300) grams of the powdered leaves of *Chromolaena odorata* were soaked in two (2) litres of water and allowed to stand for 24 hours to ensure maximum extraction. The mixture was stirred at 2 hours interval within the 24hrs extraction period. After 24 hours, the mixture was sieved using muslin cloth and filtered with Whatmann-no 1 filter paper. The filtrate was concentrated using water bath at 50℃.

Animals: Thirty-eight (38) male Wistar rats weighing between 120g and 150g were procured from Chris Experimental Farm, Awka. The rats were kept in a well aerated stainless steel cage and allowed to acclimatize for seven days before the commencement of the experimental feeding period. They were maintained under ambient conditions with normal rat diet and water provided *ad libitum*. The animals were handled in accordance with the guidelines of the ethics committee of animal research.

Induction of Hepatotoxicity: Hepatotoxicity was induced intraperitoneally in the rats using 2ml/kg of carbon tetrachloride for three consecutive days. Blood was collected by *retro orbital sinus* for haematological analysis to monitor the symptoms of hepatotoxicity before the commencement of treatment.

Animal Study Design: The rats were divided into five groups of five animals each. This study lasted for a month. All treatment groups were induced with hepatotoxicity using carbon tetrachloride. The groups will be as follows:

- Group A (Control group): This group received only food and water.
- Group B: This group of animals were induced with 2ml/kg CCl_4 and left untreated.
- Group C: This group were induced with 2ml/kg CCl_4 and treated with standard drugs (100mg/kg silymarin).
- Group D: This group were induced with 2ml/kg CCl_4 and treated with 100mg/kg extract of *C. odorata*
- Group E: This group were induced with 2ml/kg CCl_4 and treated with 300mg/kg extract of *C. odorata.*

Blood Collection

At the end of the experimental feeding period, the rats were anesthetized using chloroform after an overnight fast. Blood samples were collected via cardiac puncture using 10ml syringe and

deposited into plain sample bottles. The sera samples were obtained after centrifuging for 10 minutes at 4000 rpm and used to carry out the research analysis.

LABORATORY ANALYSIS.

LIVER FUNCTION PARAMETERS

Determination of Alanine Aminotransferase (ALT)

The ALT was assayed using the method of Reitman and Frankel (1957) as outlined in Randox Kit.

Principle: α-oxoglutarate + L-Alanine $\xrightarrow{\text{GPT}}$ L-Glutmate + Pyruvate

Alanine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine

Procedure: ALT substrate (0.5ml) was added to Test, Test Blank, Standard, and Standard Blank. The tubes were incubated for 5 minutes at 37°C. Afterwards, 0.1 mL of serum was added to the Test tube, 0.1 mL of pyruvate standard was added to the Standard tube, and 0.1 mL of distilled water was added to the Test Blank and Standard Blank tubes. All tubes were mixed thoroughly and incubated at 37°C for 30 minutes. After the incubation, 5.0 mL of 2,4-dinitrophenylhydrazine was added to all the tubes, mixed properly, and incubated at 25°C (room temperature) for 20 minutes. This was followed by the addition of 5.0 mL of 0.4N sodium hydroxide (NaOH) to all the tubes. The absorbance was read at 505 nm using a spectrophotometer, after zeroing with the appropriate blank. The results were then read off using the calibration curve provided.

Determination of Aspartate Aminotransferase (AST)

The AST was determined using the method of (Reitman and Frankel, 1957) as outlined in Randox Kit.

Principle: α-oxoglutarate +L aspertate $\xrightarrow{\text{GOT}}$ L-glutamate + oxaloacetate

AST was assayed by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure: A 0.5 ml of AST substrate was added to Test, Test Blank, Standard, and Standard Blank. This was incubated for 5 minutes at 37ºC. Afterwards, 0.1 ml of serum was added to Test, 0.1 ml of pyruvate standard was added to Standard, and 0.1 ml of distilled water was added to Test Blank and Standard Blank. They were mixed and incubated at 37ºC for 30 minutes. After the incubation, 5.0 ml of 2,4-dinitrophenylhydrazine was added to all the tubes, mixed, and incubated for 20 minutes at room temperature (25ºC).

This was followed by the addition of 5.0 ml of 0.4N NaOH to all the tubes and the absorbance was read at 505 nm after zeroing the spectrophotometer with the blank. The results were then read off using the calibration curve provided.

Determination of Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) was determined using Randox kit as recommended by Deutsche Gesellschaft fur Klinische Chemmie (GSCC)

Principle: P-nitrophenylphosphate + H_2O ALP Phosphate + Pnitrophenol (a coloured chromogen)

Procedure: To the Test, Test Blank, and Standard, 1.0 ml of alkaline buffer and phenyl phosphate substrate was added. They were incubated for 3 minutes at 37ºC. Afterwards, 0.1 ml of serum was added to Test, 0.1 ml of phenol standard was added to Standard, and 0.1 ml of distilled water was added to Test Blank. They were further incubated for another 15 minutes at 37ºC. Following the incubation, 1.0 ml of 0.5N sodium hydroxide (NaOH), 1.0 ml of 0.5N sodium hydrogen carbonate ($NaHCO_3$), 0.1 ml of 4-aminoantipyrine, and 0.1 ml of potassium ferricyanide were each added to all the tubes, mixed, and the absorbance was read immediately after zeroing the spectrophotometer with the blank at 510 nm wavelength.

Determination of Bilirubin

The concentrations of conjugated and unconjugated bilirubin were determined using the method of Jendrassik and Grof (1938) as outlined in Randox Kit.

Principle

Colorimetric method: Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin-bound bilirubin, by the reaction with diazotized sulphanilic acid.

Procedure for Direct bilirubin (D. Bil)

The sample blank and sample tubes were set for the analysis. About 200 µl of sulphanilic acid was put into the sample blank and sample tubes. Then 50 µl of sodium nitrite was added into the sample tube. Then 2000 µl of sodium chloride was added to both sample blank and sample tube. Also, 200 µl of the sample was added to both sample blank and sample tubes. The tubes were mixed and allowed to stand for 10 minutes at 25ºC. The absorbance of the sample was read at a wavelength of 546 nm against the sample blank.

Procedure for Total Bilirubin (T. Bil)

The sample blank and sample tubes were set for the analysis. About 200 µl of sulphanilic acid was put into the sample blank and sample tubes. Then 50 µl of sodium nitrite was added into the sample tube. Then 1000 µl of caffeine was added to both sample blank and sample tubes. Also, 200 µl of the sample was added to both sample blank and sample tubes. The tubes were mixed and allowed to stand for 10 minutes at 25ºC. Then 1000 µl of titrate was added to both sample blank and sample tubes. The tubes were mixed and incubated for 20 minutes at 25ºC and then, the absorbance of the sample was read at a wavelength of 578 nm against the sample blank.

KIDNEY FUNCTION PARAMETERS

Determination of Creatinine

Method used: Jaffe colorimetric method.

Principle: Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Procedure: A working reagent containing equal volumes of Reagent A (alkaline solution) and Reagent B (picric acid solution) was prepared. For each determination, 1 ml of Reagent A was mixed with 1 ml of Reagent B to give 2 ml of working reagent for control and test. Then, 0.1 ml of standard serum was added to the tube marked Standard, and 0.1 ml of the test serum was added to the tube marked Test. Afterwards, 1 ml of the working reagent was added to both tubes. The tubes were mixed, and the absorbance of the tubes was read and noted within 30 seconds. The absorbance was read for the second time at exactly 2 minutes. The tubes were read at a wavelength of 500 nm.

Determination of Urea

Method used: Berthelot method.

Principle: Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia reacts with phenol and hypochlorite in an alkaline medium to form indophenol. The intensity of the colour indicates the concentration of urea in the sample. Nitroprusside is used to catalyse the reaction. This indophenol is then measured photometrically.

Procedure: About 0.01 ml of serum was added to the test tube. Then, 0.01 ml of urea standard was added to the standard tube, and 0.01 ml of distilled water was added to the blank tube. In sequence to these additions, 0.1 ml of urea reagent 1 was added to all the tubes, mixed thoroughly, and incubated at 37ºC for 10 minutes. Afterwards, 2.5 ml of urea

reagents 2 and 3 were each added to all the tubes, mixed thoroughly, and incubated at 37ºC for 15 minutes. The absorbance was read at 540 nm after blanking the spectrophotometer with the reagent blank.

RESULTS

ACUTE TOXICITY STUDY (LD_{50}) STUDY

Table 1a: Effect of administration of low doses of aqueous extract of *C. odorata* leaves to the rats

Extract dose (mg/kg body weight)	Mortality
10	0/3
100	0/3
1000	0/3

Number of deaths per group =0;
Number of rats per group = 3.

Table 1b: Effect of administration of high doses of aqueous extract of *C. odorata* leaves to the rats.

Extract dose (mg/kg body weight)	Mortality
1600	0/1
2900	0/1
5000	0/1

Number of deaths per group =0;
Number of rats per group = 1.

The animals that were administered low doses in Table 4a (10, 100 and 1000 mg/kg bodyweight) of aqueous leave extract of *C. odorata* survived after 14 days of administration without a sign or symptom of toxicity such as restlessness, drowsiness and mortality. Another set of rats were administered high doses in Table 4b (1600,

2900 and 5000 mg/kg bodyweight) of aqueous leave extract of *C. odorata* and these set of rats did not show any sign of toxicity.

There was no death in Phase I and II, which suggests that the extract is safe for consumption. Since there was no death, the LD_{50} is estimated to be above 5000mg/kg, which means that the extract is practically non-toxic according to LD_{50} classification scale (Loomes and Hayes, 1996).

LIVER FUNCTION TEST

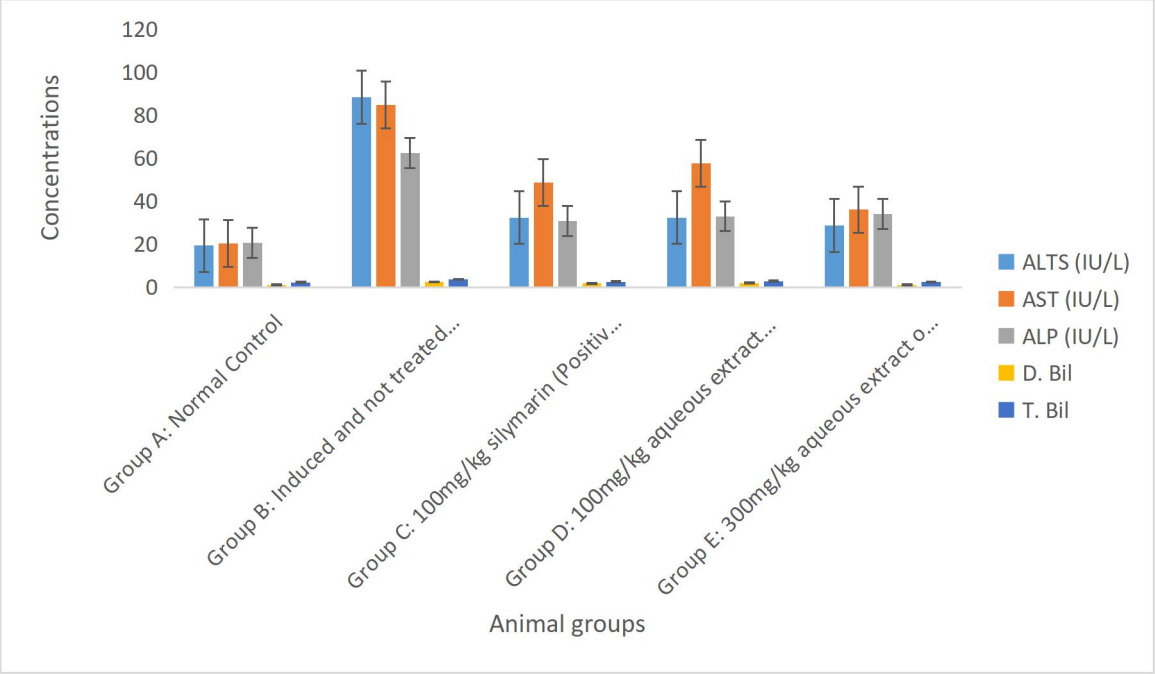

Figure 1: Effects of aqueous extract of *C. odorata* on Liver Function parameters.

Induction of hepatotoxicity in group B showed a significant increase ($p<0.05$) in the Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Direct Bilirubin (D.Bil) and Total Bilirubin (T.Bil) when compared to normal control group which was not induced (Fig. 4.6). A significant reduction ($p < 0.05$) in Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Direct Bilirubin (D.Bil) and Total Bilirubin

(T.Bil) was observed in group C treated with standard drug compared to hepatotoxicity untreated group. The Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Direct Bilirubin (D.Bil) and Total Bilirubin (T.Bil) indicated a significant decrease ($p < 0.05$) in the groups induced and treated with graded doses of the extract compared to negative control groups. On the other hand, there was an increase in Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Direct Bilirubin (D.Bil) and Total Bilirubin (T.Bil) in group D and E treated with the extracts compared to group A which was not induced.

KIDNEY FUNCTION TEST

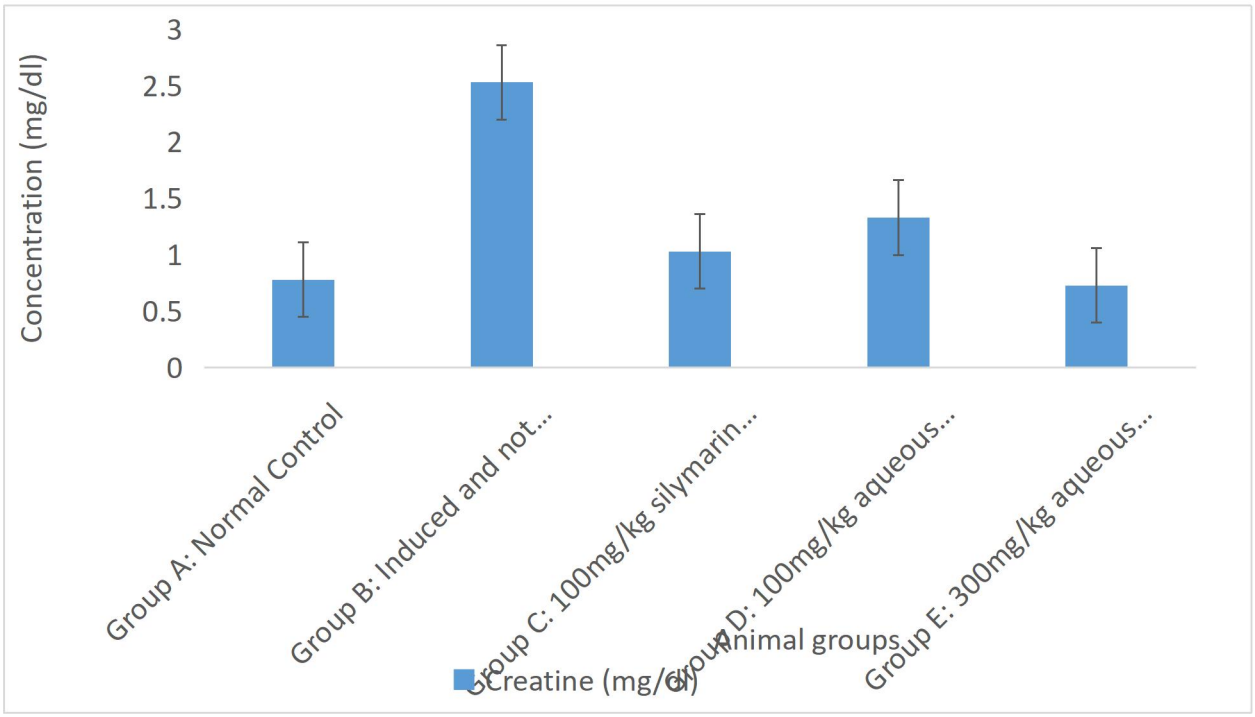

Figure 2a: Effect of aqueous extract of *C. odorata* on Kidney function parameter (Creatine).

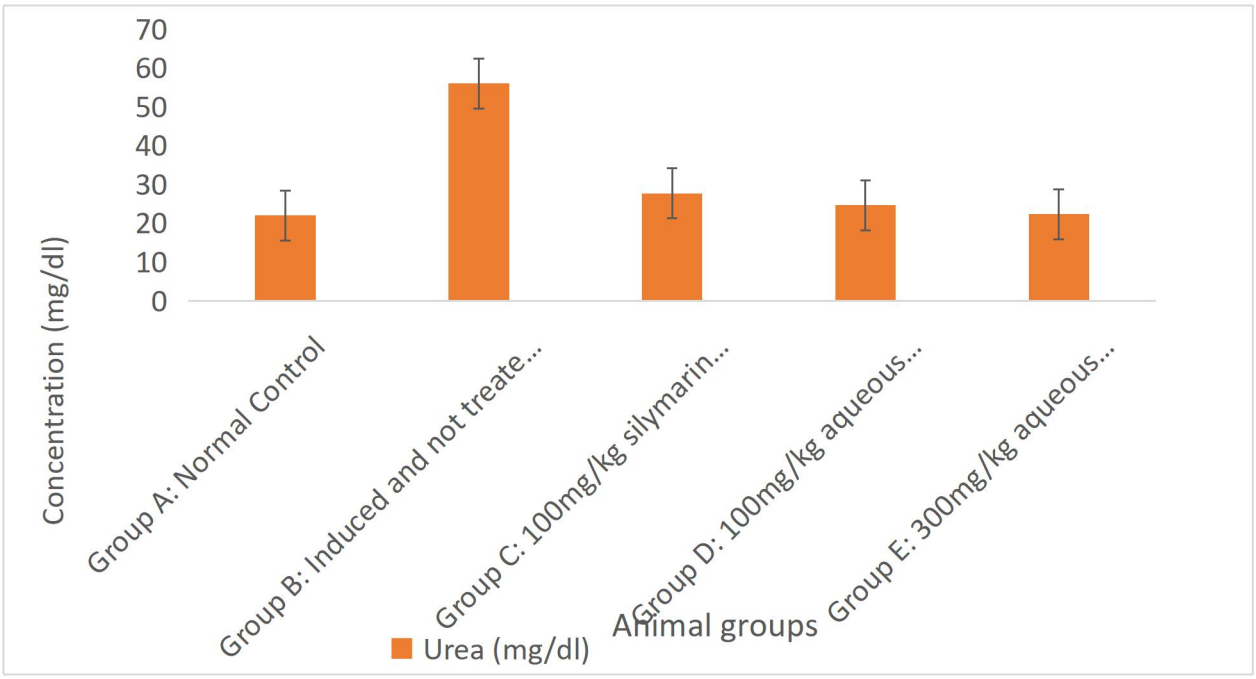

Figure 2b: Effect of aqueous extract of *C. odorata*on Kidney function parameters (Urea)

The Creatinine level (Fig. 4.7a) of group B significantly increased ($p<0.05$) when compared to normal control (group A). There was a significant decrease ($p<0.05$) in the group induced and treated with standard drug (group C) and the groups induced and treated with graded doses of the extract(group D and E) compared to negative control (group B). A slight increase in group D and E was observed compared to normal control group.

The Urea concentration (Fig. 4.7b) showed a significant increase ($p<0.05$) in group B when compared to normal control (group A). There was a significant decrease ($p<0.05$) in the group induced and treated with standard drug (group C) and the groups induced and treated with graded doses of the extract (group D and E) compared to negative control (group B). A slight increase in group D and E was observed compared to normal control group.

DISCUSSION

This study evaluated the hepatoprotective and nephroprotective effects of aqueous extract of *Chromolaena odorata* leaves in male Wistar rats subjected to carbon tetrachloride (CCl_4)-induced hepatotoxicity. The results provide promising evidence that the plant extract confers significant protection to both liver and kidney functions in a dose-dependent manner.

The absence of mortality in rats following both low and high doses of *Chromolaena odorata* leaf extracts (10, 100, 1000, 1600, 2900, and 5000 mg/kg) suggests that the plant extract may not have acute toxicity at these dose levels. The result implies that the median lethal dose (LD_{50}) of the extract could be higher than 5000 mg/kg, meaning that, in terms of acute toxicity, the extract might be relatively safe for rats at the levels tested.

The findings of liver function test demonstrated that the low levels of ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), D. Bil (direct bilirubin), and T. Bil (total bilirubin) indicate normal liver function with no significant liver injury in group. Group B, which was induced with hepatotoxicity and left untreated, showed significantly elevated levels of ALT, AST, ALP, D. Bil, and T.Bil. This suggests that the hepatotoxic agent successfully caused liver damage, leading to elevated levels of these biomarkers. ALT and AST are enzymes released into the bloodstream when liver cells are damaged, while ALP elevation may indicate bile duct obstruction or liver damage. The elevated bilirubin levels (both direct and total) indicate impaired liver function, potentially due to decreased ability of the liver to conjugate and excrete bilirubin, a waste product of hemoglobin breakdown. The groups induced with hepatotoxicity but treated with *Chromolaena odorata* extract exhibited lower levels of biomarkers (ALT, AST, ALP, D. Bil, and T. Bil) compared to Group B, suggesting that the extract may have a hepatoprotective effect. However, these biomarkers were still higher than in normal control groups, indicating that while the extract may provide some protection, it does not fully reverse the hepatotoxic effects of the induced injury. This result aligns with the study by *Mbongue et al. (2015)* which showed that *Chromolaena odorata* have antioxidant and hepatoprotective properties, which can help mitigate

oxidative stress, a major contributor to hepatotoxicity.

The result of kidney function test indicated low levels of creatinine and urea in group A, which served as the normal control group. . This is expected, as healthy kidneys efficiently filter waste products from the bloodstream, maintaining low concentrations of these markers (Xu *et al.,* 2016). Group B, which was induced with hepatotoxicity but left untreated, exhibited significantly elevated levels of both creatinine and urea. This suggests that liver damage led to kidney dysfunction. Hepatic injury can induce secondary renal impairment, commonly referred to as hepatorenal syndrome (Schrier *et al.,* 2004). The groups which were induced and treated with *C. odorata* extract showed reduced levels of creatinine and urea compared to Group B and elevated compared to Group A. The elevated level indicated that while the extract offers some protective effect, it does not fully restore renal function, while the reduction suggests that the extract may possess some protective or restorative effect on kidney function in the context of hepatotoxicity. This supports the findings of Saeed *et al*. (2018) that *C. odorata* have hepatoprotective and antioxidant properties, which may contribute to reducing kidney damage secondary to liver injury.

CONCLUSION

The findings of this study demonstrated that the aqueous extract of *C. odorata* has significant protective and therapeutic effect against hepatotoxicity. The LD_{50} determination suggests that the extract is safe at therapeutic doses. The improvement in liver and kidney function tests indicated that *C. odorata* can effectively ameliorate liver damage. These findings suggest that *Chromolaena odorata* may offer a promising natural alternative for the treatment of liver toxicity, though further studies, including clinical trials is needed to validate its efficacy and safety in humans.

REFERENCES

Ajaiyeoba, E. O., Adeyemi, O. O. and Onajobi, F. D. (2017). *Phytochemical and antioxidant activity of Chromolaena odorata leaf extract.Nigerian Journal of Natural Products and Medicine,* **21**(2): 60-66.

Drake R.L., Duparc, F., Duparc, J., Mitchell. A.H., Vogl, A.W. and J. Scott. (2015). Edition Elsevier Health Sciences, Amsterdam, ´ Netherlands. *Gray's Anatomie pour les ´etudiants*, pp. 186–199.

Gogoi, M., Kalita, P. and Sharma, D. (2020). *Evaluation of nutritional and medicinal potentials of Chromolaena odorata*. *International Journal of Plant Research*, **10**(2): 72-79.

Jendrassik, L., and Grof, P., (1938). Colorimetric method of determination of bilirubin. *Biochem Z*; **297**:81-82.

Lalith Gunasekera (2009) Invasive Plants: A guide to the identification of the most invasive plants of Sri Lanka, Colombo, p. 116–117.

Mbongue, J., Nguefack, F. and Tchoumbougnang, F. (2015). "Antioxidant and hepatoprotective activities of *Chromolaena odorata* in experimental models of liver injury." *Journal of Medicinal Plants Research, **9***(13): 391-398.

Ostapowicz, G., Fontana R.J., Schiodt F.V., Larson A., Davron J.T., Steven H.B., Timothy M., and Reish, J. (2002). Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med*., **137**: 947–954.

Phyllis, T. S. and Edson, J. B. (2019). *Antioxidant properties of Chromolaena odorataleaves inthe context of vitamin E activity*. *Journal of Ethnopharmacology.*, **132**(1), 50-58.

Rajib Ahsan, Km Monirul Islam, Musaddik, I.A. and Haque,I.E. (2009). Hepatoprotective Activity of Methanol Extract of Some Medicinal Plants Against Carbon Tetrachloride Induced

Hepatotoxicity in Albino Rats Global. *Journal of Pharmacology*, **3** (3): 116.

Reitman, S. and Frankel, S. (1957). A colorimetric method for determination of serum glutamateoxaloacetate andpyruvate transaminases. *Am.J.Clin Path*, **28**:56-61.

Robert D. (2008) Liver disease Fundamentals of Clinical Chemistry. *Dufour* pg. 676.

Saeed, A., Ahmed, M. and Al-Ansari, M. (2018). The hepatoprotective potential of *Chromolaena odorata* in animal models of liver injury. *Phytotherapy Research*, **32**(10), 1891-1900.

Suksamrarn, A., Chotipong, A., Suavansri, T., Boongird, S.,Timsuksai, P., Vimuttipong, S. and Chuaynugul, A. (2004). Antimycobacterial activity and cytotoxicity of flavonoids from the flowers of Chromolaena odorata. *Arch Pharm Res*, **5**(27): 507-51.

Teschke, R. (2018). Liver Injury by Carbon Tetrachloride Intoxication in 16 Patients Treated with Forced Ventilation to Accelerate Toxin Removal via the Lungs: A Clinical Report. *Toxics*, **6**(2) [PMC free article: PMC6027346] [PubMed: 29702608].

Vidona, W.B. and Wadioni,A. (2018). Assessment of liver histomorphology and curative effect of chloroform extract of telfairia occidentalis seed on Carbon Tetrachloride (CCL) induced liver toxicity in wistar rats. *J Biomedical Sci*. 2018; **7**(1):4-5.

Xu, M., Zhao, Z. and Li, Z. (2016). Renal function markers in clinical practice. *Clinical Chemistry and Laboratory Medicine*, **54**(7), 1032-1038.