**Ameliorative effect of *Chromolaena odorata* on Liver and Kidney Function parameters of acetaminophen-induced toxicity in rats**

**ABSTRACT**

 *Chromolaena odorata* is a medicinal plant reported to have diverse pharmacological functions including detoxifying and ameliorating potentials.The use of *Chromolaena odorata* as a medicinal plant for the treatment of different ailments has gained attention worldwide from time immemorial. The study aimed to evaluate the ameliorating effect of aqueous extract of *Chromolaena odorata* leaves on acetaminophen-induced hepatocellular and renal injury in male albino Wistar rats. The aqueous extract of *C. odorata* was prepared using conventional methods. Phytochemical and proximate of the leaves of *C. odorata* were investigated using standard methods. Theliver and kidney function analysis were carried out using several standard diagnostic methods. The result of the phytochemical analysis revealed that flavonoids (10.91±0.00)g/100g content of *C. odorata* leaves was higher followed by alkaloids (7.45±0.00)g/100g, cardiac glycosides (5.22±0.00)g/100g and saponins (4.96±0.00)g/100g. Proximate analysis showed that carbohydrate (63.34±0.00)% content was significantly higher compared to moisture (11.75±0.00)%, protein (10.15±0.00)%, fibre (5.63±0.00)%, fat (5.29±0.00)% and ash (3.85±0.00)% content. The ALT activity (43.33 ± 2.60)IU/L, AST (62.33±7.13) IU/L activity, ALP (42.00±1.53)IU/L activity, D.Bil (1.93±0.09)mg/dl and T.Bil (3.13±0.18)mg/dl concentration of the acetaminophen-induced untreated animals were significantly (*p*<0.05) higher compared with the ALT activity (21.67 ± 1.33)IU/L, AST (21.67±1.33) IU/L activity, ALP (19.33±3.76) IU/L activity, D.Bil (0.93±0.09)mg/dl and T. Bil (2.57±0.15)mg/dl concentration of the group treated with 300mg/kg bodyweight of the aqueous extract. Treatment with 100mg/kg and 300mg/kg bodyweight of the aqueous extract of *C. odorata* for a period of four weeks significantly ameliorated the alterations in the liver function and kidney function caused by acetaminophen toxicity. The extracts restored to a greater extent the damages caused by acetaminophen toxicity as was revealed from the results of the liver and kidney function tests. The extract modulates the essential biochemical parameters and organ-system functions of acetaminophen-induced toxicity in rats favourably towards recovery and improved health.

**1.0 INTRODUCTION**

The liver remains the major centre for detoxification of xenobiotics. Acetaminophen is one of the most common and available analgesic and antipyretic medications worldwide and is highly accessible because it has fewer side effects than any other analgesic or antipyretic. However, acetaminophen overdose can cause serious and lethal hepatotoxicity [1]. Bioaccumulation of acetaminophen as a result of prolonged intake can result to liver or kidney diseases. The daily maximal dose of acetaminophen is 4.0 g, and excessive doses between 7.5 g and 10.0 g can cause hepatotoxicity [2] because about 10% of acetaminophen is metabolized into the toxic metabolite N-acetylp-benzoquinone imine (NAPQI) by cytochrome P450 [3]. Large doses of acetaminophen result in severe glutathione depletion with overproduction of NAPQI, causing hepatotoxicity.

The liver is one of the vital organs, indispensable for normal physiological functions of living organisms. It plays an important role in metabolic functions such as protein, carbohydrate and fat digestion, excretion of endogenous and exogenous waste, bile secretion, urea formation, and detoxification of xenobiotics from the body [4]. Liver diseases involve the damage of liver tissues due to various pathophysiological factors resulting in mortality at high rates. Hepatotoxicity implies chemical-driven liver damage via hepato-toxins [5]. Acetaminophen, while generally safe at recommended dosages, can cause kidney damage, particularly with overdose or in individuals with certain risk factors. This damage, often manifesting as acute tubular necrosis, can lead to acute kidney injury (AKI) or even chronic kidney disease (CKD) in some cases. Excessive acetaminophen intake overwhelms the body's ability to process it, leading to the formation of a toxic metabolite (NAPQI) that damages kidney cells. Overdose depletes glutathione, a crucial antioxidant, and shifts the metabolic pathway of acetaminophen toward oxidation, which can cause cellular damage and apoptosis. Acetaminophen can increase reactive oxygen species (ROS), contributing to cell damage and kidney injury.

Acetaminophen is the leading cause of drug-induced liver injury worldwide [6]. Toxicity refers to the capacity of a chemical (toxin) to affect an organism adversely. Environmental pollutants are usually appertained to the term xenobiotics, which are chemical compounds found in but not naturally produced within an organism or a biological system. Environmental xenobiotics mostly are wastes from anthropogenic activities (e.g., agriculture, mining, industrialization, urbanization, chemical spills, etc.), which can be grouped as pharmaceutical drugs, environmental pollutants, food additives, pesticides, carcinogens, antioxidants, hydrocarbons, and many more.

Drug-induced liver injury is a cause of acute and chronic liver disease caused specifically by medications and the most common reason for a drug to be withdrawn from the market after approval. The global burden of hepatotoxicity affects over fifty million people worldwide [7]. Depending upon origin, there are different types of liver diseases which include acute liver failure, hepatitis, liver cirrhosis, non-alcoholic fatty liver disease [8]. Physiological factors such as age, gender, nutrition, pregnancy, and genetic factors including deficiency in certain enzymes may worsen liver injury. The most common drugs induce liver injury include antibiotics, isoniazid and nonsteroidal anti-inflammatory drugs. As liver plays vital role in the metabolism of drugs resulting in active and inactive metabolites, hence it is the target of acetyl-para-aminophenol or acetaminophen poisoning and oxidative damage due to its toxic metabolite [9].

Paracetamol poisoning, also known as acetaminophen poisoning, is caused by excessive use of the medication paracetamol (acetaminophen). Most people have few or non-specific symptoms in the first 24 hours following overdose. These symptoms include feeling tired, abdominal pain, or nausea. Acetaminophen is a commonly used analgesic and antipyretic drug that is found in a number of combined prescriptions, including Tylenol with codeine and Hycotab [10]. Although its effectiveness and safety were confirmed at recommended doses, the acetaminophen overdose causes hepatotoxicity that leads to acute liver failure [11]. According to the acute liver failure Study Group in the United States, acetaminophen-associated toxicity contributes to approximately 46% of all cases of acute liver failure in adults, which far exceeds idiosyncratic drug-induced liver injury by more than fourfolds ([12,13].

Accumulating evidence has highlighted that acetaminophen-induced oxidative stress and mitochondrial dysfunction are the fundamental factors in the pathogenesis of acetaminophen-associated liver injury; thus, N-acetyl cysteine, a scavenger of reactive oxygen species, is considered a standard therapeutic option for acetaminophen overdose [14]. However, due to the narrow therapeutic window, severe adverse effects, and rapid disease progression, the therapeutic efficacy of N-acetyl cysteine is still limited [15]. For patients at the advanced stage, liver transplantation is the only way to improve survival outcomes [16]. Therefore, new treatments that are superior to NAC in terms of therapeutic efficacy and safety are required in clinical practice.

Recently, herbal medicine was found to be a promising therapeutic approach for acute liver injury. Several herbal components were reported to have the same therapeutic effect as N-acetyl cysteine [17,18,19]. The effectiveness of mecicinal plants in the treatment of illnesses has been proven in previous studies [20,21,22,23,24]. Medicinal plants exhibit its therapeutic effect in chemical induced diseases which can be as a result of its numerous phytochemical [25,26,27], mineral, vitamin [28] and proximate compositions [29]. Despite supplements and approved drugs available in the market, there is still a need for the development of novel interventions for the treatment and prevention of drug-induced liver diseases. Thus, greater attention needs to be given to drug-induced liver disease for reducing the rate in future for successful use of different drugs. In developing nations, about 80% of the population is dependent on traditional medicines which are reported to be a combination of phytochemical and herbal plants for curing various diseases. The use of these natural remedies to treat diseases helps in reducing the toxic metabolites with minimum cost [30,31]. The included phytochemicals and plant extracts were reported to be hepatoprotective which upon further research might become versatile therapeutic approaches for treating liver toxicity. The contribution of different species of plant parts to health status of man cannot be over emphasized. Various plants in Nigeria have been identified to have therapeutic potential. They become important when their functions are considered in the human body [32]. Most of these plants are used in treatment of some pathological conditions.

*Chromolaena odorata* (L), is an ornamental plant usually considered to be one of the top 100 most invasive environmental weeds of wastelands, roadsides and other exposed areas in the world [33]. This flowering shrub is native to North and Central America, and was later introduced to parts of Asia, Africa and Australia. *C.* *odorata* is also known by various other names such as Armstrong’s weed, baby tea, bitter bush, butterfly weed, Christmas bush, devil weed, eupatorium, Jack in the bush, king weed, paraffin bush, paraffin weed, Siam weed, turpentine weed and triffid weed. .It possesses insecticidal properties and is used as a green manure. It is also used for the preservation of dead bodies [34]. *Chromolaena odorarata* leaf. found throughout the world especially in the pacific region under different names; Siam weed, devil weed, French weed, hagonoy, co hoy, in Indonesia known as Ki Rinyuh and si koko [35]. The mentioned herb is an important weed plant that extend its territory from America to Asian countries like Indonesia, India, China, Bangladesh, Thailand and other [36]. *Chromolaena odorata* Leaf is being used traditionally as medicinal properties.

The extract from the fresh leaves has been used for the treatment of various ulcers. 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 also for the treatment of leech bites, indigestion and skin infection [37]. The numerous medicinal uses of *C. odorata* geared our interest in investigating its ameliorative property in treating and managing hepatocellular and renal injury caused by acetaminophen toxicity.

**2.0 METHODS**

**2.1 Sample Collection and Identification**

*C. Odorata* leaves was gotten from a farm at Umunya, Anambra State. The leaves of *C. odorata* were identified by a taxonomist in the department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka. The herbarium number is NAUH – 73D.

**2.2 Sample preparation**

The leaves were cut into smaller pieces and air-dried at room temperature for 4 weeks. They were pulverized to powder form using a corona grinder. The powdered leaves were exhaustively extracted with distilled water for 24 hours. The aqueous filtrate was concentrated using a Vacuum rotary evaporator (N-100, Eyla, Tokyo, Japan) at 40ºC to a dark brown mass and the residues was transferred to separate bottles and stored in a refrigerator until use.

**2.3 Phytochemical Analysis**

Phytochemical analysis was carried out to identify the secondary metabolites present in the extracts using standard methods [38].

**2.3.1 Oxalate Determination by Titration Method**

This determination involves three major steps: digestion, oxalate precipitation and permanganate titration

**Digestion**

1. 2g of sample was suspended in 190ml of distilled water in a 250ml volumetric flask.
2. 10ml of 6m HCl was added and the suspension digested at 1000c for 1 hour.
3. It was cooled, and then make up to 250ml mark before filtration.

**Oxalate precipitation**

Duplicate portions of 125ml of the filtrate were measured into beakers and four drops of methyl red indicator added. This was followed by the addition of NH4OH solution (dropwise) until the test solution changes from salmon pink colour to a faint yellow colour (pH4-4.5). Each portion was then heated to 900C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 900C and 10ml of 5% CaCl2 solution was added while being stirred constantly. After heating, it was cooled and left overnight at 250C. The solution was then centrifuge at 2500rpm for 5 minutes. The supernatant is decanted and the precipitate completely dissolved in 10ml of 20% (v/v) H2S04 solution.

**Permanganate titration**

At this point, the total filtration resulting from digestion of 2g of flour was made up to 300ml. Aliquots of 125ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized KMnO4 solution to a faint pink colour which persists for 30s. The calcium oxalate content was calculated using the formula

T x (Vme)(Df) x 105 (mg/100g)

 (ME) x Mf

Where T is the titre of KMn04(ml), Vme is the volume-mass equivalent (i.e. 1ml of 0.05m KMn04 solution is equivalent to 0.00225g anhydrous oxalic acid). Df is the dilution factor Vt/A (2.4 where Vt is the total volume of titrate (300ml) and A is the aliquot used (125ml), ME is the molar equivalent of KMn04 in oxalate (KMn04 redox reaction) and Mf is the mass of sample used [39].

**2.3.2 Alkaloids Determination**

Five grams (5g) of the sample were weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added, covered and allowed to stand for 4 hours at 250C. This was filtered with filter paper No. 42, and the filtrate was concentrated using a water bath (Memmert) to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected and washed with dilute NH4OH (1% ammonia solution). Then, filtered with pre-weighed filter paper. The residue on the filter paper is the alkaloid, which is dried in the oven (precision electrothermal model BNP 9052 England) at 800c. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed [40].

Calculation:

%weight of alkaloid = weight of filter paper with residue – weight of filter paper x 100

 Weight of sample analyzed

**2.3.3 Flavonoids Determination**

Ten (10g) of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatmann filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a waterbath and weighed to a constant weight [41].

Calculation:

% flavonoids = (weight of crucible + residue) – (weight of crucible ) x 100

 Weight of sample analyzed

**2.3.4 Determination of Saponin**

Exactly 5g of the sample was put into 20% acetic acid in ethanol and allowed to stand in a waterbath at 500C for 24 hours. This was filtered, and the extract was concentrated using a waterbath to one-quarter of the original volume. Concentrated NH4OH was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage [40].

Calculation:

%saponin content = (weight of filter paper + residue) – (weight of filter paper) x 100

 Weight of sample analyzed

**2.3.5 Cardiac Glycosides Determination**

Wang and Filled method was used. To 1ml of extract was added 1ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1ml of 5% aqueous NaOH. It was boiled for 2 minutes

(until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 500c till dryness and weight of the filter paper with residue was noted.

The cardiac glycoside was calculated in %.

Calculation:

%cardiac glycoside = (weight of filter paper + residue) – (weight of filter paper) x 100

 Weight of sample analyzed

**2.3.6 Tannin Determination by Follins Dennis Titration**

The Follins Dennis titrating method as described by Pearson [42] was used. To 20g of the crushed sample in a conical flask was added 100ml of petroleum ether and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100ml of 10% acetic acid in ethanol for 4hrs. The sample was then filtered and the filterate collected.

25ml of NH4OH were added to the filter ate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some of the NH4OH still in solution. The remaining volume was measured to be 33ml. 5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.1M Na0H using phenolphthalyneasndicator until a pink end point is reached. Tannin content was then calculated in % (C1V1 = C2v2) molarity.

**Calculation**

Data

C1 = conc. of Tannic Acid

C2 = conc. Of Base

V1 = Volume of Tannic acid

V2= Volume of Base

Therefore C1 = C2V2

 V1

% of tannic acid content = C1  x 100

 Weight of sample analyzed

**2.3.7 Phytate Determination**

Phytate contents were determined using the method of [43] as adopted by Lucas and Markakes [44]. 0.2g of each of the different processed corns was weighed into different 250ml conical flasks. Each sample was soaked in 100ml of 2% concentrated HCL for 3hr, the sample was then filtered. 50ml of each filtrate was laced in 250ml beaker and 100ml distilled water added to each sample. 10ml of 0.3% ammonium thiocynate solution was added as indicator and titrated with standard iron (111) chloride solution which contained 0.00195g iron per 1ml.

 Phytic acid = Titre value x 0.00195 x 1. 19 x 100

 Wt of sample

**2.3.8 Phenol Determination**

The quantity of phenol was determined using the spectrophotometer method. The plant sample is boiled with 50ml of (CH3CH2)2O for 15 mins. 5ml of the boiled sample is then pipetted into 50ml flask, and 10ml of distilled water is added. After the addition of distilled water, 2ml of NH4OH solution and 5 ml of concentrated CH3(CH2)3CH2OH was added to the mixture. The samples was made up to the mark and left for 30 mins to react for colour development and measured at 505nm wavelength using spectrophotometer.

**2.3.9** **Determination of Anthocynanin in the Water of Life Using The Gravimetric Method of Harborne** [38]

**Principle**

Acid hydrolysed sample when filtered reacts with ethylacetate to enable extraction of anthocyanin. Upon addition of amyl alcohol, anthocyanin was extracted and after drying, the percent composition was determined in relation to weight of original sample gravimetrically.

**Procedure**

Five (5.0)g of the powdered sample (water of life) was boiled in 100ml of 2MHCl for 30 minutes. The hydrolysate was filtered using whatmann filter paper. The filterate was transferred into separation funnel and equal volume of ethylacetate added, mixed and allowed to separate into two layers. The ethylacetate layer was recovered while the aqueous layer was discarded.

The extract was dried over a steam bath. The dry extract was then treated with 10ml of concentrated Amylalcohol to extract the anthocyanin. After filtration, the alcohol extract was dried. The weight of anthocyanin was determined and expressed as percentage of original sample.

Calculation (%) = Weight of Anthocyanin x 100

 Weight of original sample

**2.3.10 Determination of Steroid Content**

1.0g of the powdered sample was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was filtered, and the filtrate eluted with 0.1N ammonium hydroxide solution. 2ml of the eluent was put in a test tube and mixed with 2ml of chloroform. 3ml of ice-cold acetic anhydride was added to the mixture in the flask. 2 drops of (200mg/dl) standard sterol solution was prepared and treated as described for test as blank. The absorbance of standadrd and test was measured,zeroing the spectrophotometer with blank at 420nm.

Calculation (mg/100ml) Absorbance of test x Conc of std

 Absorbance of std.

**2.4.0 Proximate Analysis**

**2.4.1 Moisture Content** [45]

Procedure

* A petri dish was washed and dried in the oven
* Approximately 1-2g of the sample was weighed into petri dish
* The weight of the petri dish and sample was noted before drying
* The petridish and sample were put in the oven and heated at 1050C for 2 hrs. The result noted and heated another 1hr until a steady result is obtained and the weight was noted
* The drying procedure was continued until a constant weight was obtained

% moisture content = W1-W2 x 100
 Weight of sample

Where W1 = weight of petridish and sample before drying

W2 weight of the petridish and sample after drying.

**2.4.2 Carbohydrate Determination**

(Differential method)

100 – (%Protein + %Moisture + %Ash + %Fat + %Fibre)

**2.4.3 Ash Content**

(AOAC, 1990)

Principle: The ash of foodstuff is the inorganic residue remaining after the organic matter has been burnt away. It should be noted, however, that the ash obtained is not necessarily of the composition as there may be some from volatilization.

Procedures

* Empty platinum crucible was washed, dried and the weight was noted.
* Approximately 1- 2g of sample was weighed into the platinum crucible and placed in a muffle furnace at 5500c for 3 hours.
* The sample was cooled in a dessicator after burning and weighed.

 Calculations

% Ash content =

 W3 -W1 x 100

 W2 -W1 1

Where

W1 = weight of empty platinum crucible

W2 = weight of platinum crucible and sample before burning

W3 = weight of platinum and ash.

**2.4.4 Crude Fibre (AOAC 1990)**

**Procedure:**

1. Defat about 2g of material with petroleum ether (if the fat content if more than 10%)
2. Boil under reflux for 30 minutes with 200ml of a solution containing 1.25g of H2SO4 per 100ml of solution
3. Filter the solution through linen
4. Wash with boiling water until the washing is no longer acidic.
5. Transfer the residue to a beaker and boil for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml
6. Filter the final residue through a thin but close pad of washed and ignited asbestos in a Gooch crucible
7. Dry in an electric oven and weigh
8. Incinerate, cool and weigh

The loss in weight after incineration x 100 is the percentage of crude fibre.

% crude fibre = Weight of fibre x 100

 Weight of sample

**2.4.5 Crude Fat**

**Soxhlet Fat Extraction Method**

This method is carried out by continuously extracting food with non-polar organic solvent such as petroleum ether for about 1 hour or more.

**Procedure:**

1. Dry 250ml clean boiling flasks in oven at 105 - 1100C for about 30 minutes.
2. Transfer into a dessicator and allow to cool
3. Weigh correspondingly labeled, cooled boiling flasks.
4. Fill the boiling flasks with about 300ml of petroleum ether (boiling point 40 - 600c)
5. Plug the extraction thimble lightly with cotton wool
6. Assemble the soxhlet apparatus and allow to reflux for about 6 hours
7. Remove thimble with care and collect petroleum ether in the top container of the set – up and drain into a container for re – use.
8. When flask is almost free of petroleum ether, remove and dry at 1050C - 1100C for 1hour.
9. Transfer from the oven into a dessicator and allow to cool; then weigh.

% fat = weight of flask + oil - wt of flask x 100

 weight of sample

**2.4.6 Crude Proteins** [45]

**Principle**: The method is the digestion of the sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia. This is retained in the solution as ammonium sulphate. The solution is made alkaline and then distilled to release the ammonia. The ammonia is trapped in dilute acid and then titrated.

**Procedures**

* Exactly 0.5g of sample was weighed into a 30ml kjehdal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stoppered and shaken. Then 0.5g of the kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared.
* The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling was made up to 100ml with distilled water was added to avoid caking and then 5ml was transferred to the kjedahl distillation apparatus, followed by 5ml of 40% sodium hydroxide.
* A 100ml receiver flask containing 5ml of 2% boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methlene blue was placed added under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution and distillation commenced immediately until 50 drops gets into the receiver flask, after which it was titrated to pink colour using 0.01N hydrochloric acid.

Calculations

% Nitrogen =Titre value x 0.01 x 14 x 4

% Protein = % Nitrogen x 6.25

**2.5.0 ACETAMINOPHEN-INDUCED HEPATOCELLULAR AND RENAL INJURY**

**2.5.1 STUDY DESIGN AND ANIMAL GROUPING**

Twenty-five (25) rats will be randomized into 5 groups of five rats each and used for the study. The grouping is as follows:

Group A: Normal Control

Group B: 42.9 mg/kg Acetaminophen (Negative Control)

Group C: Standard drug -100 mg/kg Silymarin (Positive Control)

Group D: 100 mg/kg aqueous extract of *C. odorata*

Group E: 300 mg/kg aqueous extract of *C. odorata*

**2.6 LIVER FUNCTION TEST**

Serum biochemical indices routinely estimated for liver functions were analysed. They include: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct and total bilirubin. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer’s instruction.

**2.7 KIDNEY FUNCTION TEST**

Serum biochemical indices routinely estimated for kidney functions were analysed. The kidney function parameters analysed include creatinine and urea. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer’s instruction.

**2.8 STATISTICAL ANALYSIS**

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests to determine if a significant difference exists between the mean of the test and control groups. The limit of significance was set at *p*<0.05.

**3.0** **RESULTS OF LIVER FUNCTION TEST**

**3.1 RESULTS OF PHYTOCHEMICAL ANALYSIS**

The mean ± SEM of the phytochemical composition of C. odorata leaves are shown in figure 1. The flavonoids (10.91 ± 0.00)g/100g content was highest followed by alkaloids (7.45 ± 0.00) g/100g, cardiac glycosides (5.22 ± 0.00)g/100g, saponins (4.96 ± 0.00)g/100g, anthocyanins (3.26 ± 0.00)g/100g, tannins (3.08 ± 0.00) g/100g, steroid (0.94 ± 0.00)g/100g, phenol (0.78 ± 0.00)g/100g, phytate (0.55 ± 0.00) g/100g, oxalate (0.25 ± 0.00)g/100g, cyanogenic glycosides (0.06 ± 0.00)g/100g and haemaglutin (0.03 ± 0.00)g/100g. Haemaglutin was the lowest in quantity detected in the leaf sample.

**Figure 1:** Phytochemical analysis of *C. odorata* leaves

**3.2 RESULTS OF PROXIMATE ANALYSIS**

The mean ± SEM of the proximate composition of C. odorata are shown in figure 2. The carbohydrate content (63.34 ± 0.00)% is highest followed by moisture content (11.75 ± 0.00)%, protein content (10.15 ± 0.00)%, fibre (5.63 ± 0.00)%, fat (5.23 ± 0.00)% and ash content (3.85 ± 0.00)%.

**Figure 2:** Proximate analysis of *C. odorata* leaves

**3.3 RESULTS OF LIVER FUNCTION TEST OF ACETAMINOPHEN-INDUCED TOXICITY**

The result of the baseline and follow-up of the effect of administration of aqueous extract of *C. odorata* leaves on alanine transaminase (ALT) activity of acetaminophen-induced hepatocellular injury in rats was expressed as mean ± SEM and is represented in figure 3. The baseline values of the ALT were recorded before the commencement of four weeks treatment and checked again after the four weeks treatment to ascertain the effect of the extract on the ALT activity. Induction of hepatocellular injury using acetaminophen caused a significant increase in the ALT activity of the rats compared to the normal control group left uninduced. Treatment with 100 and 300mg/kg bodyweight of the aqueous extract significantly reduced the ALT activity of the treated groups compared with the untreated.

**Figure 3:** Effect of aqueous extract of *C. odorata* on alanine transaminase activity of acetaminophen-induced toxicity in Wistar rats.

The result of the baseline and follow-up of the effect of administration of aqueous extract of *C. odorata* leaves on aspartate transaminase (AST) activity of acetaminophen-induced hepatocellular injury in rats was expressed as mean ± SEM and is represented in figure 4. The baseline values of the AST were recorded before the commencement of four weeks treatment and checked again after the four weeks treatment to ascertain the effect of the extract on the AST activity. Induction of hepatocellular injury using acetaminophen caused a significant increase in the AST activity of the rats compared to the normal control group left uninduced. Treatment with 100 and 300mg/kg bodyweight of the aqueous extract significantly reduced the AST activity of the treated groups compared with the untreated.

**Figure 4:** Effect of aqueous extract of *C. odorata* on aspartate transaminase activity of acetaminophen-induced toxicity in Wistar rats.

The result of the baseline and follow-up of the effect of administration of aqueous extract of *C. odorata* leaves on alkaline phosphatase (ALP) activity of acetaminophen-induced hepatocellular injury in rats was expressed as mean ± SEM and is represented in figure 5. The baseline values of the ALP were recorded before the commencement of four weeks treatment and checked again after the four weeks treatment to ascertain the effect of the extract on the ALP activity. Induction of hepatocellular injury using acetaminophen caused a significant increase in the ALP activity of the rats compared to the normal control group left uninduced. Treatment with 300mg/kg bodyweight of the aqueous extract significantly reduced the ALP activity of the treated groups compared with the untreated.

**Figure 5:** Effect of aqueous extract of *C. odorata* on alkaline phosphatase activity of acetaminophen-induced toxicity in Wistar rats.

The result of the baseline and follow-up of the effect of administration of aqueous extract of *C. odorata* leaves on the direct bilirubin concentration of acetaminophen-induced hepatocellular injury in rats was expressed as mean ± SEM and is represented in figure 6. The baseline values of the direct bilirubin concentration were recorded before the commencement of four weeks treatment and checked again after the four weeks treatment to ascertain the effect of the extract on the direct bilirubin concentration. Induction of hepatocellular injury using acetaminophen caused a significant increase in the direct bilirubin concentration of the rats compared to the normal control group left uninduced. Treatment with 100mg/kg and 300mg/kg bodyweight of the aqueous extract significantly reduced the direct bilirubin concentration of the treated groups compared with the untreated.

**Figure 6:** Effect of aqueous extract of *C. odorata* on direct bilirubin concentration of acetaminophen-induced toxicity in Wistar rats.

The result of the baseline and follow-up of the effect of administration of aqueous extract of *C. odorata* leaves on total bilirubin (T.BIL) concentration of acetaminophen-induced hepatocellular injury in rats was expressed as mean ± SEM and is represented in figure 7. The baseline values of the T.BIL concentration were recorded before the commencement of four weeks treatment and checked again after the four weeks treatment to ascertain the effect of the extract on the T.BIL concentration. Induction of hepatocellular injury using acetaminophen caused a significant increase in the T.BIL concentration of the rats compared to the normal control group left uninduced. Treatment with 100 and 300mg/kg bodyweight of the aqueous extract significantly reduced the T.BIL concentration of the treated groups compared with the untreated.

**Figure 7:** Effect of aqueous extract of *C. odorata* on total bilirubin concentration of acetaminophen-induced toxicity in Wistar rats.

**3.4 RESULTS OF KIDNEY FUNCTION TEST**

The result of the effect of administration of aqueous extract of *C. odorata* leaves on the creatinine concentration of acetaminophen-induced hepatocellular injury in rats was expressed as mean ± SEM and is represented in figure 8. Induction of hepatocellular injury using acetaminophen caused a significant increase in the creatinine concentration of the rats compared to the normal control group left uninduced. Treatment with 100mg/kg and 300mg/kg bodyweight of the aqueous extract significantly reduced the creatinine concentration of the treated groups compared with the untreated.

**Figure 8:** Effect of aqueous extract of *C. odorata* on creatinine concentration of acetaminophen-induced toxicity in Wistar rats.

The result of the effect of administration of aqueous extract of *C. odorata* leaves on the urea concentration of acetaminophen-induced hepatocellular injury in rats was expressed as mean ± SEM and is represented in figure 9. Induction of hepatocellular injury using acetaminophen caused a significant increase in the urea concentration of the rats compared to the normal control group left uninduced. Treatment with 100mg/kg and 300mg/kg bodyweight of the aqueous extract did not cause any significant difference (*p*<0.05) in the urea concentration of the treated groups compared with the untreated.

**Figure 9:** Effect of aqueous extract of *C. odorata* on urea concentration of acetaminophen-induced toxicity in Wistar rats.

**4.0 Discussion**

Acetaminophen-induced toxicity is on the increase due to the fact that paracetamol is an over-the-counter drug in most countries. This has made it possible for a good number of the populace to abuse the drug thereby increasing the risk of hepatocellular and renal diseases. Apart from orthodox medicine, the use of herbal drugs in the treatment of liver and kidney disorder is a common practice in many developing countries. Acetaminophen is an analgesic and an antipyretic drug. It has ubiquitous applications and is readily available worldwide with no less great potential for toxic effects as compared to other common pain relief medications like ibuprofen and aspirin ([46]. *Chromolaena odorata* is one of the important medicinal plants with high therapeutic value. Its therapeutic value is because of various chemical constituents present in it. This is also attributed to its high content of beneficial phytochemicals which play a great role in its therapeutic properties. This study revealed that *C. odorata* leaf contains important phytochemicals and nutrients which made it possible for its ameliorative properties in acetaminophen-induced hepatocellular injury in Wistar rats.

The phytochemical analysis revealed that *C. odorata* is highly rich in flavonoids, moderately rich in alkaloids, cardiac glycosides, saponins, tannins and anthocyanins with little content of phytate, oxalate steroid, phenol and heamaglutin. The aqueous leaf extract showed a high antioxidant activity and ameliorative effect on the hepatocellular injury in rats which could be as a result of flavonoids and alkaloids which helps to protect plants from oxidative stress. The flavonoid content was higher than other phytochemicals detected in the leaf of C. odorata. The presence of flavonoids in substantial amount in C. odorata is an indication of medicinal benefits due to its antioxidant properties and increased activity of the enzymes that detoxify carcinogens [14].

The proximate analysis showed that carbohydrate content (63.34±0.00) was highest in the leaf followed by moisture (11.75±0.00) and then protein (10.15±0.00). According to Igboh *et al.,* [47] the carbohydrate content in dry leaf sample is (50.82±0.00) which is lower than the value detected in our research. This could be as a result of the location where the sample was collected. The *C. odorata* leaf also contains fibre, ash and fat. The high carbohydrate content of *C. odorata* shows that the leaf can be a good source of energy when consumed or used as a vegetable in food.

Acetaminophen administration caused a markedly significant increase (p<0.05) in the liver function parameters (ALT, AST, ALP, D.BIL and T.BIL). The liver is the organ responsible for the metabolism and detoxification of chemical compounds. In a related development, Asomugha *et al.* [48] found that the activities of liver function enzymes correlate with the synthesis of the enzymes in the liver and are important indicators of liver tissue derangement [49]. The findings further stated that the activities of these enzymes are a measure of liver integrity (Asomugha *et al.,* 2014). Rats treated with graded doses of the aqueous extract of *C. odorata* showed reduced activity of the liver function enzymes.

Ingestion of toxic amounts of acetaminophen can cause acute kidney injury (AKI) because of acute tubular necrosis at the proximal tubule, and habitual acetaminophen use may result in chronic kidney disease (CKD) and end-stage renal disease (ESRD) from chronic interstitial fibrosis or papillary necrosis [50]. Excessive amounts of acetaminophen and NAPQI are excreted by the kidney and may contribute to AKI. Furthermore, the associated oxidative stress can lead to direct nephrotoxicity [51]. However, evidence that habitual use of acetaminophen alone increases the risk of ESRD has not been clearly demonstrated. A population-based cohort study reported that single-substance acetaminophen may be safe for patients with advanced CKD at stage 4 to 5 without an adverse effect on progression of CKD [52]. Aqueous extract of *C. odorata* leaf significantly reduced (*p*<0.05) creatinine and urea concentrations when compared with the induced untreated control. These findings show that *C. odorata* can be used to manage and treat kidney disorder resulting from acetaminophen toxicity.

**Conclusion**

In conclusion, acetaminophen can cause liver and kidney damage through various mechanisms, including overdose, metabolic shifts, oxidative stress, and tubular damage. While therapeutic doses are generally safe, individuals with risk factors or those who take excessive amounts of acetaminophen are at higher risk of developing kidney injury

Based on the findings from this work and correlation with other works, it is evident that acetaminophen influenced alteration of liver and kidney parameters biochemical parameters of the experimental animals. The presence of phytoconstituents in *C. odorata* leaves extracts make the plant useful for treating different liver and kidney ailments and have a potential of providing useful drugs of human use in the treatment of acetaminophen-induced toxicity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**REFERENCES**

1. Woo YP. Controversies in acetaminophen nephrotoxicity. Kidney Research and Clinical Practice. 2020; 39(1):4-9.
2. Zed PJ, Krenzelok EP. Treatment of acetaminophen overdose. American Journal of Health-System Pharmacy. 1999; 56:1081-1091.
3. Moyer AM, Fridley BL, Jenkins GD. Acetaminophen-NAPQI hepatotoxicity: a cell line model system genomewide association study. Toxicol Sci. 2011; 120:33-41.
4. Borude P, Bhushan B, Gunewardena S, Akakpo J, Jaeschke H, Apte U. Pleiotropic Role of p53 in Injury and Liver Regeneration after Acetaminophen Overdose. *American Journal of Pathology.* 2018;188(6):1406–1418.
5. Schuppan D, Nezam A. Liver Cirrhosis. Lancet. 2008; 307:838-851.
6. Chang L, Xu D, Zhu J, Ge G, Kong X, Zhou Y. Herbal Therapy for the Treatment of Acetaminophen-Associated Liver Injury: Recent Advances and Future Perspectives. *Frontal Pharmacology.* 2020; 11:313.
7. Bruha R., Dvorak K, Petrty J. Alcoholic liver disease. *World Journal of Hepatotoxicity*

2012;4:81-90.

1. Huo Y., Yin S, Yan M., Win S, Aung Than T, Aghajan, M, et al. Protective role of p53 in

acetaminophen hepatotoxicity. *Free Radical Biological Medicine. 2017;* 106:111–117.

1. Mobasher MA, Gonzalez-Rodriguez A, Santamaria B, Ramos S, Martin MA, Goya L. (2013). Protein tyrosine phosphatase 1B modulates GSK3beta/Nrf2 and IGFIR signaling pathways in acetaminophen-induced hepatotoxicity. *Cell Death Disease.* 2013; 4:e626.
2. Aminoshariae A., Khan A. (2015). Acetaminophen: old drug, new issues. *Journal of Endocrinology.* 2015; 41(5):588–593.
3. Bunchorntavakul C, Reddy KR. (2018). Acetaminophen (APAP or N-Acetyl-p-Aminophenol) and Acute Liver Failure. *Clin. Liver Dis.* 2018; 22 (2), 325–346.
4. Ostapowicz, G., Fontana, R. J., Schiodt, F. V., Larson, A., Davern, T. J., Han, S. H., et al. (2002). Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann. Int. Med.* 2002; 137(12):947–954.
5. Lee WM. Drug-induced acute liver failure. *Clinical Liver Disease.* 2013: 17(4), 575–586.
6. Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. *Drug Metabolism Review.* 2012; 44(1):88–106.
7. Du K, Ramachandran A, Weemhoff JL, Chavan H, Xie Y, Krishnamurthy P. Editor’s Highlight: Metformin Protects Against Acetaminophen Hepatotoxicity by Attenuation of Mitochondrial Oxidant Stress and Dysfunction. *Toxicological Sciences.* 2016; 154(2):214–226.
8. Craig DG, Lee A, Hayes PC, Simpson KJ. Review article: the current management of acute liver failure. *Alimental Pharmacology and Therapeutics.* 2010; 31(3):345–358.
9. Mukazayire MJ, Allaeys V, Buc-Calderon P, Stevigny C, Bigendako MJ, Duez P. Evaluation of the hepatotoxic and hepatoprotective effect of Rwandese herbal drugs on in vivo (guinea pigs barbiturate-induced sleeping time) and in vitro (rat precision-cut liver slices, PCLS) models. *Experimental Toxicology and Pathology.* 2010; 62(3):289–299.
10. Tien YH, Chen BH, Wang-Hsu GS, Lin WT, Huang, JH, Lu YF. (2014). Hepatoprotective and antioxidant activities of *Glossogyne tenuifolia* against acetaminophen-induced hepatotoxicity in mice. *American Journal of Chinese. Medicine.* 2014; 42(6):1385–1398.
11. Enemali MO, Asogwa KK, Ezeigwe OC. Ezenwelu OC. The Cushioning Effect of Extracts of *Amaranthus Viridis* Linn (Green Amaranth) on Acetaminophen-Induced Hepatotoxicity. *International Journal of Research And Scientific Innovation*. 2024; 11(5)1117-1128.
12. Ezeigwe OC, Ononamadu CJ, Enemchukwu BN, Umeoguaju UF, Okoro JC. Antidiabetic and antidiabetogenic properties of the aqueous extracts of *Azadirachta indica* leaves on alloxan-induced diabetic wistar rats. *International Journal of Biosciences*. 2015; 7(2): 116-126.
13. Ononamadu CJ, Ihegboro GO, Owolarafe TA, Salawu K, Fadilu M, Ezeigwe OC, Oshobu ML. Nwachukwu FC. (2019). **Identification of potential antioxidant and hypoglycemic compounds in aqueous-methanol fraction of methanolic extract of *Ocimum canum* leaves. *Analytical and Bioanalytical Chemistry Research.* 2019;**6(2):431-439.
14. Iloanya EL, Nwodo OFC, Ezeigwe OC, Ngwu OR, Nwobodo VOG, Obayuwana EA. Antidiabetic Property of a Fraction of the Methanol Extract of the Seeds of *Abrus precatorius* in Alloxan-induced Diabetic Rats. *Journal of Complementary and Alternative Medical Research*. 2021; 13(2): 27-38.
15. Enemchukwu BN, Ezeigwe OC, Orinya OF, Nwali UI, Chigbo CM. Iloanya EL. Phytochemical evaluation and Antimicrobial potential of methanol extracts of mistletoe (*Loranthus micranthus*) leaves grown on cola tree (*Cola nitida*) and oil bean tree (*Pentaclethra macrophylla*). *Journal of Medicinal Plants Studies*, 2021; 9(2):28-32.
16. Onuabuchi NA, Ezeigwe OC, Agbo CA. Evaluation of Toxicity effects of Ethanol Extract of Fruit Rind of *Cucumis metuliferus* on the Biochemical Hematological and Histological Parameters of Albino Rats. *Journal of Complimentary and Alternative Medical Research*. 2022; 18(3): 15-27.
17. Enemor VHA, Ogbodo UC, Nworji OF, Ezeigwe OC, Okpala CO, Iheonunekwu GC. Evaluation of the Nutritional Status and Phytomedicinal Properties of Dried Rhizomes of Turmeric (*Curcuma longa*). *Journal of Biosciences and Medicine*. 2020; 8:163-179.
18. Ezeigwe OC, Okpala CO, Ogana J, Akonam NE, Okwuchukwu AB, Nnadi NN, Anekwe OJ. and Nwobodo VOG. Comparative Phytochemical and Nutritional Profiles of *Ficus capensis* and *Cnidoscolus aconitifolius* Leaves. *International Journal of Research and Innovation in Applied Science*. 2020; 5(1):16-21.
19. Anekwe-Nwokeaku, J. O., Morah, E. J., Ezeigwe, O. C., Obi, A. I., Ezeudu, E. C., Nwankwo, N. V. Extensive Study on the Pharmaceutical Constituents of the Leaves and Roots of *Costus afer* (ker). *J. Chem. Lett*. 2024; 5:165-185.
20. Ani ON, Ujah II. Ezeigwe OC. Phytochemical and Nutritional Profile of *Ficus capensis* Stem collected from Agbani, Enugu, Southeast Nigeria. *Asian Plant Research Journal*, 2024; 12(6): 27-36.
21. Igwilo IO, Iwualla LC, Igwilo SN, Agbara ACI, Okpara CO, Ezeigwe CO. Proximate analysis and Antinutrient Composition of Fresh and Dried Fruits of *Morinda lucida*. *The Bioscientist*. 2029; 6(1):31-39.
22. Enemchukwu BN, Onwuatuegwu TTC, Ofomatah AC, Ezeigwe OC, Orinya O F, Iloanya E L, Okeke CM, Onyedinma EI. Biochemical and Haematological Effects of *Telfairia occidentalis* and *Amaranthus viridis* extracts on Anaemic Rats. *Journal of Applied Sciences*, 2022; 22(3):144-151.
23. Ezeigwe OC, Okpala CO, Enemali MO, Iloanya EL, Chigbo CM, Okeke CB, Okafor MC. Effect of *Citrus aurantifolia* juice on bodyweight and haematological indices of wistar rats. *African Journal of Food Science*, 2022; 16(6), 151-159.
24. [Adegoke](https://scholar.google.com/citations?user=eUdIht4AAAAJ&hl=en&oi=sra) OA, [Idowu](https://scholar.google.com/citations?user=Nd0qPTIAAAAJ&hl=en&oi=sra) OS, Olaniyi AA. Characterization and partial purification of antioxidant component of ethereal fractions of *Aframomum danielli*. *World Journal of Chemistry.* 2006; 1:1-5.
25. Chakraborty AK, Sujit R. Umesh KP. *Chromolaena odorata* (L.): An overview. *Journal of Pharmacy Research.* 2011; 43:573–576.
26. Ukwueze ES, Duru OM. Shorinwa OA. Evaluation of the cutaneous wound healing activity of solvent fractions of *Chromolaena odorata* linn. *Indo American Journal of Pharmaceutical Research.* 2013; 3:3316–3323.
27. Vaisakh MN, Pandey A. (2012). The invasive weed with healing properties: A review on Chromolaena odorata. *International Journal of Pharmaceutical Science Research.*2012; 3:80–83.
28. Luwun P. Control of invasive *Chromolaena odorata;* An evaluation in some land use types in Kwazulu Natal-South Africa. *Thesis submitted to International Institute for Geoinformation Science and Earth Observation.* 2002; 7(1): 21–39.
29. Panyaphu K, On TV, Sirisa-Ard P, Srisa-Nga P, ChansaKaow S, Nathakarnkitkul S. Medicinal plants of the Mien (Yao) in Northern Thailand and their potential value in the primary healthcare of postpartum women. *Journal of Ethnopharmacology* 2011; 135:226–237.
30. Harborne JB. Phytochemical methods – a guide to modern techniques of plant analysis. 2nd edn. Springer Netherlands.1984; pp 4–16.
31. Harborne JB. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, Third Edition, Champman and Hall, New York. 1995.
32. ObadoniBO.Ochuko PO. Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Homeostatic Plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science.* 2001; 8:203-208.
33. Boham, B.A. and Kocipal-Abyazan, R. (1994). Flavonoids and Condensed Tannins from Leaves of awaiian vaccinium. *Vaticulatum and V. calycinlum*. *Pacific Science*. **48**:458-463.
34. Pearson DT. Methods of Food Analysis. Chemic al Analysis of Food 7thed. Churchill Livingstone Edinburgh. 1976; **p**. 324.
35. Young and Greaves. Laboratory Handbook of Methods of Food Analysis. Leonard Hill, London. 1940.
36. Lucas GM. Markakas. Phytic acid and other phosphorus compounds of bean (*Phaseolus vulgaris*).*Journal of Agricultural and Food Chemistry*. 1975;23: 13-15.
37. AOAC. “Official Methods of Analysis, Association of Official Analytical Chemists”, 17th ed., Washington DC USA. 2005.
38. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS, Schiødt FV, Ostapowicz G, Shakil AO, Lee WM; Acute Liver Failure Study Group. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology (Baltimore, Md.) 2005; 42(6): 1364–72.
39. Igboh MN, Ikewuchi J. Ikewuchi C. Chemical profile of *Chromolaena odorata* leaves. *Pakistan Journal of Nutrition*. 2009; 8(5):521-524.
40. Asomugha RN, Okafor PN, Ijeh II. Toxicological evaluation of aqueous leaf extract of *Chromolaena odorata* in male Wistar albino rats. *Journal of Applied Pharmaceutical* *Science*. 2013; 3: 89-92.
41. Asomugha RN, Okafor PN, Ijeh II. (2014). Hepatic effects of aqueous extract of *Chromolaena odorata* in male Wistar albino rats. *Pharmacology online*. 1: 127-136.
42. Blantz RC. Acetaminophen: acute and chronic effects on renal function. Am J Kidney Dis

1996;28(1):S3-S6.

1. Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. J Med Toxicol. 2008;4:2-6.
2. Evans M, Fored CM, Bellocco R. Acetaminophen, aspirin and progression of advanced chronic kidney disease.Nephrol Dial Transplant 2009; 24:1908-1918.