Original Research Article

The rhizome of *Curculigopilosa* exerts hepatoprotective and nephroprotective effects in rats exposed to acetaminophen toxicity

ABSTRACT

Aims: The exposition of drug-induced hepatotoxicity and nephron injury is highly uneven; some range from asymptomatic but elevated liver and kidney parameters to meteoric liver and kidney damage. The medicinal plants have been exploited for fighting toxicity, but their mechanisms remain unexplored. This purpose of this study was to see how an aqueous extract of *Curculigopilosa* (*C. pilosa*) rhizome affected the hepato- and nephro-toxicity is caused by 750 mg/kg and 1000 kg/mg body weight doses of acetaminophen (APAP) in rats.
Methodology: Thirty (30) female rats used for this study were divided into six groups (n = 5). Group A served as control. *C. pilosa* (300 mg/kg body weight) was given to Group B. Group C was administered a 750 mg/kg body weight dose of APAP. Group D was administered a 750 mg/kg body weight APAP dose plus 300 ml/kg body weight of an aqueous extract of *C. pilosa*. A 1000 mg/kg body weight APAP dose was given to Group E. Group F was administered APAP (dose 1000 mg/kg body weight) plus an aqueous extract of *C. pilosa* (300 mg/kg body weight). The serum concentrations of urea, creatinine, chloride, potassium, sodium, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were analyzed using standard methods.
Results: The APAP-induced toxicity caused a significant (*P*=.05) increase in the levels of ALT, AST, ALP, creatinine, urea, potassium, sodium, and chloride, but treatment with an aqueous extract of *C. pilosa* rhizome significantly (*P*=.05) decreased the levels of these markers compared to the control.
Conclusion: The aqueous extract of *C. pilosa* rhizome has hepatoprotective and nephroprotective effects against APAP-induced toxicity.

Keywords: Hepatoprotective, Nephrotoxicity, Acetaminophen, Curculigopilosa

1. INTRODUCTION

The naturally occurring phytoconstituents compounds (alkaloids, phenols, saponins, carbohydrates, terpenoids, steroids, flavonoids, and tannins, among others) in plants are derived from different plant parts and are primarily responsible for the treatment of different illnesses and diseases brought on by toxicity [1]. Acetamionophen (APAP) causes hepatotoxicity and nephrotoxicity. APAP is commonly used as an analgesic and antipyretic drug to relieve mild and chronic pain and reduce fever when taken in a normal, prescribed dose. Its metabolism happens within liver microsomes, where it forms water by conjugating with glucuronate and sulfate to be eliminated from the liver and blood through the bile and urine [2-4]. At prescribed doses, APAP is oxidized to the electrophile N-acetyl-p-benzoquinone imine (NAPQI), a toxic compound, by microsomal cytochrome P450. The detoxification of NAPQI is done with reduced glutathione (GSH) by conjugation to form the

acetaminophen-glutathione conjugate (APAP-SG). The prolonged use of APAP causes APAP-SG, which, collectively with GSH depletion, stirs up a series of damaging reactions leading to acute liver damage (ALD) and cell death [2,4]. In addition to ALD, the long-term use of APAP is metabolized in the kidney to produce NAPQI that is deacetylated to produce a nephrotoxic product, 4-aminophenol (PAP), resulting in nephrotoxicity such as acute kidney injury (AKI). The APAP-induced toxicity results in oxidative stress, inflammation, lipid peroxidation, protein oxidation, modification and inhibition of enzyme activity, mitochondrial dysfunction, and endoplasmic reticulum stress [2,3,5-7].

The search for a drug with hepato-nephro-protective and/or hepato-nephro-regenerative functions that is reliable, without side effects, and readily available is difficult [8,9]. Therefore, it is pertinent to consider the benefits of medicinal plants' several bioactive compounds. *Curculigopilosa* (*C. pilosa*) is a tuberous rhizome herb belonging to the Amaryllidaceae family, widely distributed in the tropical West Africa, and, known as "epakun" in the Yoruba traditional medicine of southwestern Nigeria. It is used in the treatment of hernia, infertility, genital infections, sexually transmitted infections, etc. [10,11]. In addition to its use in the production of baby food and sorghum beer in West Africa [12]. The mechanism of action in carrying out these treatments remains enigmatic. The present study is designed to investigate the hepatoprotective and nephroprotective properties of APAP in toxicity-induced rats.

2. METHODOLOGY

2.1 Plant Collection and Extraction

C. pilosa rhizomes were purchased from a Lusada market, Ado-odo/ota L.G.A., Ogun State, in South Western part of Nigeria, and identified by the Department of Botany, Lagos State University, Ojo, Lagos State, Nigeria. The rhizomes were thoroughly washed and grounded, and 200 g were boiled in 1000 ml of clean water for 30 minutes. The solution was filtered using Whatman's No.1 filter paper, and the filtrate was concentrated using a rotary evaporator. The extract was kept in the refrigerator prior to analysis. The dried mass yielded 62.5 g.

2.2 Experimental animals

Thirty (30) healthy female Wistar rats weighing 85–100 g were kept in the animal house of the Department of Biochemistry, Lagos State University, Ojo, Lagos, Nigeria, between April 2018 and July 2018. The animals were acclimatized for two (2) weeks, fed *ad libitum* daily with commercial rat feed (product of Animal care, Lagos, Nigeria), and clean water, and kept under a constant 12-hour light and dark cycle. A single dose of acetaminophen suspension was administered by the oral route, at a dose of 750 mg/kg b.wt (groups C - D) and 1000 mg/kg b.wt (groups E - F). Preliminary pilot studies showed that *C. pilosa* extract was not toxic up to 300mg/kg body weight, according to the method of Karigidi and Olaiya [13]. The experimental protocols were conducted in accordance with the international guiding principles of laboratory animal care [14] and were approved by the Animal Ethical Committee of the Department of Biochemistry, Lagos State University; Ojo, Nigeria.

2.3 Experimental design

The rats were randomly divided into six (6) groups of five rats each as shown below:

Group A = Control

Group B = Rats treated with 300 mg/kg body weight of *C. pilosa* aqueous extract

Group C = Rats treated with 750 mg/kg body weight acetaminophen

Group D = Rats treated with 750 mg/kg body weight acetaminophen plus 300 mg/ kg body weight of *C. pilosa* aqueous extract

Group E = Rats treated with 1000 mg/kg body weight acetaminophen

Group F = Rats treated with 1000 mg/kg body weight acetaminophen plus 300 mg/ kg body weight of *C. pilosa* aqueous extract

The extracts were administered orally for 14 days using intragastric tube. After 14 days, the rats were fasted overnight and euthanized under light anesthesia. The blood was collected without anticoagulant sample bottles for serum.

2.4 Blood sample collection and biochemical analysis

Blood samples for biochemical analysis were collected into plain tubes and allowed to clot. Serum was separated with a high speed macro-centrifuge at 3,500 rpm for 10 minutes and stored for the analysis of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea nitrogen, creatinine, and electrolytes (sodium, potassium, and chloride), were determined using commercial kits, respectively.

2.5 Statistical analysis

Data were expressed as mean ± SEM, and analyzed by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences version 17.0 (SPSS, Chicago, Illinois). Values that gave a $p < 0.05$ were taken to be statistically significant.

3. RESULTS AND DISCUSSION

The variables that were assessed in the current investigation, including creatinine, urea, chloride, potassium, sodium, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) concentrations, provided an indirect evaluation of hepatic and renal function. In the liver, ammonia, a waste product and highly toxic substance, is detoxified through conversion to urea, which is nontoxic and water-soluble and excreted through urine by the kidneys, but a high concentration of urea is also toxic, hence it is considered a marker of hepatic or renal functional status [15,16]. Likewise, creatinine is an excretion product of muscle activity, and its elimination is entirely by the kidneys, where it is freely filtered in renal glomeruli, by the tubular component, which is a good indicator of renal-glomerular function [16]. Both urea and creatinine are end products of body metabolism and essentially reflect the glomerular filtration rate (GFR) [15,17]. APAP-induced hepatotoxicity and nephron injury are usually characterized by elevated serum levels of urea and creatinine, which are significant biomarkers of renal dysfunction [18,17]. In the present study, urea and creatinine concentrations, as indicated in Table 1, were elevated in APAP-treated rats (750 and 1000 mg/kg b.wt), suggesting kidney damage. This is in accordance with the administration of acrylamide [16], and lipopolysaccharide [6]. The treatment with an aqueous extract of C. pilosa rhizome significantly ($p<0.05$) decreased the APAP's high dose-induced toxicity. This indicates that the aqueous extract of C. pilosa ameliorates renal function against kidney damage. It may be due to the phytoconstituents present in the C. pilosa aqueous extract that inhibit the accumulation of urea and creatinine, respectively. This is in agreement with the research of Imo and Uhegbu [19], and Karigidi and Olaiya [13].

Guideline for Reporting *P* values:

P is always italicized and capitalized.

i) Correct expression: (*P* = .05). Wrong Expression: (*P*< .05), unless *P*< .001.

ii) The *P* value should be expressed to 2 digits whether or not it is significant. If *P*< .01, it should be expressed to 3 digits.

iii) When rounding, 3 digits is acceptable if rounding would change the significance of a value (eg, *P* = .049 rounded to .05).

iv) Expressing *P* to more than 3 significant digits does not add useful information since precise *P* values with extreme results are sensitive to biases or departures from the statistical model.

v) Reporting actual *P* values avoids this problem of interpretation. *P* values should not be

listed as not significant (NS) since, for meta-analysis, the actual values are important and not providing exact *P* values is a form of incomplete reporting.
vi) Do not use 0 before the decimal point for statistical values *P*, alpha, and beta because they cannot equal 1.

Tables & figures should be placed inside the text. Tables and figures should be presented as per their appearance in the text. It is suggested that the discussion about the tables and figures should appear in the text before the appearance of the respective tables and figures. No tables or figures should be given without discussion or reference inside the text.

Tables should be explanatory enough to be understandable without any text reference. Double spacing should be maintained throughout the table, including table headings and footnotes. Table headings should be placed above the table. Footnotes should be placed below the table with superscript lowercase letters. Sample table format is given below.

Table 1. Effects of *C. pilosa* aqueous extract on kidney functions of acetaminophen-induced toxicity

Treatment dose	Creatinine Conc. (mg/ml)	Urea Conc. (mg/ml)
Control	0.56±0.15[a]	25.74±2.64[a]
C. pilosa(300 ml/kg b. wt) aq. extr.	0.47±0.11[b]	23.71±1.41[b]
750 mg/kg b. wt APAP	2.86±0.05[c]	60.97±7.07[c]
750 mg/kg b.wt APAP + *C. pilosa*(300 ml/kg b. wt) aq. extr.	1.29±0.22[d]	33.92±4.28[d]
1000 mg/kg b.wt APAP	3.02±0.29[e]	65.10±2.31[e]
1000 mg/kg b.wt APAP+ *C. pilosa*(300 ml/kg b. wt) aq. extr.	1.42±0.31[f]	37.14±4.86[f]

Values are represented as Mean values ± Standard error of means, n=5. Rows of the same compartment carrying different letters of the alphabet are significantly different from each other (P= .05). APAP = acetaminophen (paracetamol), b. wt = body weight, aq. extr. = aqueous extract, Conc.= concentration

Overdose of APAP causes similar tubular effects to non-steroidal anti-inflammatory drugs via inhibition of prostaglandin synthesis, vasoconstriction, and activation of the renin-angiotensin-aldosterone system, causing electrolyte changes and or disturbances [20]. The stability of the blood's sodium, potassium, and chloride contents is a reliable sign of how well the kidneys and heart are working. An increase in electrolytes (potassium, sodium, and chloride) is the hallmark of APAP-induced toxicity. The serum electrolytes, sodium (Na^+), chloride (Cl^-), and potassium (K^+), are presented in Table 2. It was observed that, in the APAP-induced groups, there is a significant ($p<0.05$) increase in the concentration of Na^+, Cl^-, and K^+ compared to other groups. The intoxication of APAP leads to the breakdown of endogenous tissue and as a result, the derangement of serum electrolytes that occurs [21]. Toxic and overdose of APAP induces nephron damage, forms serum osmolality of the electrolytes (Na^+, Cl^-, and K^+), and significantly elevates these parameters, which may be due to renal hemodynamic compromise and tubular dysfunction [22,23]. The administration of an aqueous rhizome extract of *C. pilosa* significantly decreased the electrolytes, thereby protecting any of the alterations. This is in accordance with the research of Imo and Uhegbu [19]. A decrease in sodium lowers blood pressure in hypertensive individuals because there is linked between sodium and blood pressure. Likewise, potassium in the intracellular fluid, has been reported to be among the protective electrolytes against hypertension. The decrease effect of aqueous rhizome extract of *C. pilosa,* is an indication that membrane

structure may possibly be protected or stabilized, as observed with *Gongronemalatifolium*Benth leaf extract by Imo and Uhegbu [19].

Table 2. Effects of *C. pilosa* aqueous extract on serum electrolytes of acetaminophen-induced toxicity

Treatment dose	Potassium Conc. (mEg/L)	Sodium Conc. (mEg/L)	Chloride Conc. (mEq/L)
Control	3.69±0.25[a]	133.17±2.66[a]	73.13±6.98[a]
C. pilosa(300 ml/kg b. wt) aq. extr.	3.24±0.26[b]	130.10±3.94[b]	72.79±7.15[b]
750 mg/kg b.wt APAP	6.61±0.71[c]	150.96±2.39[c]	117.61±2.73[c]
750 mg/kg b.wt APAP + *C.pilosa*(300 ml/kg b. wt) aq. extr.	5.47±0.35[d]	143.89±4.93[d]	83.67±6.14[d]
1000 mg/kg b.wt APAP	7.74±0.25[e]	157.29±1.94[e]	140.12±3.65[e]
1000 mg/kg b.wt APAP + *C.pilosa*(300 ml/kg b. wt) aq. extr.	6.32±0.49[f]	138.15±0.59[f]	129.72±2.14[f]

Values are represented as Mean values ± Standard error of means, n=5. Rows of the same compartment carrying different letters of the alphabet are significantly different from each other (P= .05). APAP = acetaminophen (paracetamol), b. wt = body weight, aq. extr. = aqueous extract, Conc.= concentration

The liver is the vital organ that is highly sensitive to toxic agents, and its functions include detoxification of deleterious materials, regulation of numerous metabolic processes, and maintenance of body homeostasis [24,25]. The elevation of serum hepatic biomarker enzymes (AST, ALT, and ALP) is an indication of hepatocellular damage, which reveals liver enzyme leakage into the blood stream [8,26,27]. The results in Table 3 showed a significant ($p<0.05$) increase in ALT, AST, and ALP liver enzymes in the APAP-induced groups compared to the control group. This results in liver damage as a result of the leakage of these hepatic enzymes into the circulation. These liver enzyme concentrations were significantly ($p<0.05$) lower in the *C. pilosa*aqueous extract-treated groups. The report confirms previous findings by Lakshmi *et al*. [24]. The reversal of these enzymes with *C. pilosa* aqueous extract is evidence of the protection of cellular membrane integrity and tissue damage against APAP-induced damage, which may be due to the presence of the phytoconstituents [28-30].

Table 3. EEffects of *C. pilosa* aqueous extract on liver function test biomarkers of acetaminophen-induced toxicity

Treatment dose	Alanine transaminase Conc. (U/L)	Aspartate transaminase Conc. (U/L)	Alkaline phosphatase Conc. (U/L)
Control	29.64±2.59[a]	37.00±1.09[a]	23.88±0.26[a]
C. pilosa(300 ml/kg b. wt) aq. extr.	25.75±0.71[b]	30.56±0.98[b]	21.981±0.68[b]
750mg/kg b.wt APAP	59.45±2.51[c]	49.04±1.21[c]	21.50±0.47[c]
750mg/kg b.wt APAP + *C. pilosa*(300 ml/kg b. wt) aq. extr.	50.59±2.49[d]	25.44±1.87[d]	18.39±0.39[d]
1000mg/kg b.wt APAP	82.42±2.35[e]	74.50±1.79[e]	25.68±0.42[e]
1000mg/kg b.wt APAP + *C. pilosa*(300 ml/kg b. wt) aq. extr.	64.04±3.46[f]	62.18±2.17[f]	23.91±0.22[f]

Values are represented as Mean values ± Standard error of means, n=5. Rows of the same compartment carrying different letters of the alphabet are significantly different from each other (P= .05). APAP = acetaminophen (paracetamol), b. wt = body weight, aq. extr. = aqueous extract, Conc.= concentration

4. CONCLUSION

The oral administration of *C. pilosa* aqueous extract recovered the liver from hepatotoxicity and the kidney from nephrotoxicity, according to this study. The protective effect of the extract may be due to the presence of bioactive compounds that lead to hepatoregenerative and nephroprotective capacities. Further studies are needed to investigate the molecular targets involved in the hepatoregenerative and nephroprotective effects.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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