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Phytochemistry and Hepatoprotective Effect of Ethanolic Leaf Extract of *Corchorus olitorius* on Carbon Tetrachloride Induced Toxicity

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Authors' contributions

This work was carried out in collaboration between all authors. Author OFU designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SSI and CSA managed the analyses of the study. Authors IRU, CSA and OFU managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The liver serves as a processing factory, redistribution center and a receiving depot of the body. In the light of these roles, the effect of ethanol leaf extract of *Corchorus olitorius* on CCl_4 induced liver damage in wistar rats was assessed. CCl_4 (0.5ml/kg) was used to induced hepatotoxicity. 25-albino rats of wistar strain (120-150g) were used for the study and were divided into five groups of five rats per group. Group A serves as normal control and was given distilled water of treatment equivalence, group B serves as a negative control and was treated with CCl_4 and olive oil as a vehicle at the ratio of 1:1. Group C, D, and E were induced with CCl_4 and the extract administered through oral gavages at scalar doses of 500mg/kg, 750mg/kg and 1g/kg daily. The administration lasted for 15 days period after which the animals were sacrificed. Serum enzyme assay revealed that there was a marked reduction in the elevated activity of the hepatic enzymes viz alanine aminotransferase (ALT) A (71.2±3.27), B (98.8±2.61), C (78.0±3.74), D (74.8±2.77), and 74.6±1.95) respectively. Similar trend was observed for aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels when compared with the controls (*P*=.05).

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More so, the result revealed that the extract lowers serum albumin, no significant change in billirubin but increased total protein levels in all treated groups when compared with the controls (*P*=.05). Furthermore, it showed a significant decrease (*P*=.05) in White blood cell count (WBC), and platelet but no significant decrease (*P*=.05) in packed cell volume (PCV) and Hb. From the above findings, it is obvious that the ethanolic leaf extract of *Corchorus olitorius* could have hepatoprotective property.

Keywords: Corchorus olitorius; liver; carbon tetrachloride; hepatoprotective property; photochemistry.

1. INTRODUCTION

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries that has always been associated with cultural behavior, traditional knowledge and considering its bioactive constituent, which have been reported safe, without any adverse side effects especially when compared with synthetic drugs. Thus, a search for new drugs with better and cheaper substitutes from plant origin is a natural choice. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body [1]. Herbal medicine is the major stay of about 75-80% of the world population, mainly in the developing countries, for primary health care due to a better cultural acceptability, better compatibility with human body and few side effects [2].

Plant material have been known to be used in the management of degenerative diseases (such as mental illness, microbial infection, cancer) which is one area in which so many people in developing countries depend on herbal medicine [3-6].

General adverse health effects associated with human exposure to carbon tetrachloride (CCl_4) include cardiovascular diseases, developmental abnormalities (teratogenesis), neurologic and neurobehavioral disorders, diabetes, hearing loss, fibrosis, lung cancer, hematological disorders and black foot disease [7-9]. In humans, CCl_4 is known to cause cancer of the skin [10], lungs, bladder, liver and kidney [7,9,11]. However, strategies have been proposed in an attempt to control the spread of these diseases. The search for new ways to treat them stimulates the investigation of cheap and effective natural compounds as an alternative treatment of the aforementioned diseases [12,13]. The incidence of lung cancer and fibrosis of the liver in human due to advances in industrial activities, which have resulted in the discharge of CCl_4 into the environment, has led to the development of a number of available treatment options. However, due to insufficient information from literatures on the effect of *Corchorus olitorius* on hematological and biochemical parameters, this study aimed at determining the hepatoprotective effect of *C. olitorius (Ateve)* as claimed in folk medicine.

1.1 Brief Description of the Plant

According to a reviewed work by Hamzah and coworkers [14], *Corchorus olitorius (*Linn) is a leafy vegetable that belongs to the family Tiliaceae, and commonly called jute mallow in English and “ewedu” in the southwestern Nigeria. It is an animal herb with a slender stem and an important green leafy vegetable in many tropical area including Egypt, Sudan, India, Bangladesh, in tropical Asia such as Philippine and Malaysia, as well as in tropical Africa,

Japan, the Caribbean and Cyprus [15]. The plant is widely grown in the tropics for the viscosity of its leaves. The leaves (either fresh or dried) are cooked into a thick viscous soup or added to stew or soup and are rich sources of vitamins and minerals [16]. Nutritionally, *C. olitorius* on an average contain 85-87g H_2O, 0.7g oil, 5g carbohydrate, 1.5g fiber, 250-266mg Ca, 4.8mg Fe, 1.5mg 300010 vitamin A, 0.1mg thiamine, 0.3mg riboflavin, 1.5mg nicotinamide, and 53-100mg ascorbic acid per 100g [17]. In West African countries including Ghana, Nigeria and Sierra Leone, the vegetable is cultivated for the stem bark, which is used in the production of fiber (Jute), and for its mucilaginous leaves, which are also used as food vegetable [18]. The leaf extract of the plant is also employed in folklore medicine in the treatment of gonorrhea, pain, fever and tumor [19].

It is reportedly consumed as healthy, vegetable in Japan because of its rich contents of carotenoids, vitamin B1, B2, C and E, and minerals [20]. Its leaves and roots are eaten as herbal medicine in South East Asia [19]. In some part of Nigeria, leaves' decoction used for treating iron deficiency, folic acid deficiency, as well as treatment of anemia. Leaves also act as blood purifier [21] and the leaf twigs is used against heart troubles [22] while cold leaf infusion is taken to restore appetite and strength, leaves used for ascites, pains, piles, tumors, gonorrhea and fever [23].

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *C. olitorius* were obtained from the Mkar hills around University of Mkar, Benue State, Nigeria. The plant was identified and authenticated at the Federal College Forestry Jos, Plateau State.

2.2 Preparation of the Plant Extract

Fresh leaves of *C. olitorius* was collected and air-dried for 21days until constant weight was obtained. They were pulverized using a blender machine and sieved to obtain the powdered form. Forty grams (40g) was macerated in 250ml of 80% ethanol, shaken for 10 minutes and then kept in refrigerator for 24 hours at 4℃ to achieve maximum extraction [24]. The solution was filtered using Whatman No.1 filter paper and the filtrate concentrated in water bath at 40℃. The dried extract was later weighed and reconstituted in distilled water to the required dosage for administration.

2.3 Experimental Animals

Albino rats were obtained from the animal holding unit, Department of Chemical Sciences University of Mkar, Mkar. The animals were allowed to undergo acclimatization period of seven days and were housed in a wooden cage with good ventilation. They were kept at room temperature 28±2℃ and relative humidity 70% with 12 hours natural light and dark cycle. The rats were allowed free access to standard feeds bought from Vital Feeds, Gboko, Benue State and supplied with portable water. The experimental protocol was followed as approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of the European Convention for the Protection of Vertebrate animals and other scientific purposes – ETS-123 [25].

2.4 Experimental Design

25 male rats (weighing 120-150g) were selected for study. They were randomly divided into five groups (A, B, C, D and E) with their respective tails marked for easy identification. The animals in their various groups exception of group A (which serves as normal control treated with feed and water only) were treated with CCl_4 intraperitoneally using olive oil as a vehicle in the ratio of 1:1 (0.5ml/kg body weight) to induce hepatotoxicity. After 24 hours of the induction of hepatic injury, the animals in group B which serve as negative control group received feed and drinking water daily by gavages for 15 days while the remaining three test groups (C, D and E) were treated with leaf extract of *C. olitorius* on a daily dosage of 500, 750, and 1000mg/kg body weight respectively for 15 days by using oral cannula. Previous study revealed that *C. olitorius* at high doses of 2500, 5000 and 7500mg/kg body weight had no apparent toxic and lethal effects on the animals, which probably indicate that the extract has high safety index [26].

2.5 Phytochemical Screening (Quality Test)

The phytochemicals such as flavonoids, tannins, steroids, phlobatannins, saponins, terpenoids, cardiac glycosides and alkaloids were identified by chemical method [27], and as modified by Harbome (1996) and Sofowora [28,29].

2.6 Determination of Hematological and Biochemical Parameters

Whole blood was collected from the heart by cardiac puncture using sterile syringe and needle. The whole blood samples were put in ethylene diamine tetra acetate (EDTA) treated sample tubes. The packed cell volume or the haematocrit and White blood cell count (WBC) was determined by the method of Baker and Silverton [30], Hemoglobin (Hb) concentration was determined according to Jain (1986), using the cyanomethemoglobin method [31], while platelets were determined by following the method of Mitruka and Rawnsley [32].

Biochemical assay was carried out as follows: Billirubin content was measured by the method described by Jendrassik and Grof [33], Total proteins assay was conducted according to Tietz [34], while serum albumin level was examined by the method described by Grant, [35].

2.7 Determination of Serum Enzyme Assay

Alanine transaminase (ALT) and Aspartate transaminase (AST) activities were assayed using the method of Reitman and Franke [36] and Alkaline Phosphatase (ALP) serum level was estimated by the principle of Tietz, [34]. All the above biochemical parameter was determined in the plasma using the Randox kits by Cypress diagnostics (Belgium).

2.8 Statistical Analysis of Data

Data obtained was expressed as Mean±Standard Deviation and analyzed using the Analysis of Variance 'ANOVA, F-ratio' [37] and student's t' test where applicable. Values at P=.05 and P<.001 were regarded as significant in comparison with appropriate controls.

3. RESULTS

3.1 Phytochemicals of *Corchorus olitorius* Leaf Extract

The phytochemical analysis of the extract revealed the presence of tannins, steroids, saponin, terpenoids, cardiac glycosides and alkaloids as shown in Table 1.

Table 1. Phytochemical Composition of the crude extract of *Corchorus olitorius*

Phytochemicals	Crude Extracts
Flavonoids	-
Glycosides	+
Tannins	+
Steroids	+
Alkaloids	+
Phlobatannins	-
Saponins	+
Terpenoids	+

Where: (+) indicates present; (-) indicates not present

The result of ethanolic leaf extract of *C. olitorius* on some hematological, biochemical, and enzyme assay of wistar rats are presented in Tables 2, 3, and 4 respectively based on 500mg, 750mg and 1g/kg body weight of the animals.

Table 2. Effect of crude extract of *Corchorus olitorius* on serum enzyme activities of CCl_4 induced hepatotoxicity in wistar rats

Treatment	Dose	Enzyme Activity		
C. olitorius	CCl_4/kg	AST (u/l)	ALT (u/l)	ALP (u/l)
A (Normal control)		72.8±1.05	71.2±3.27	24.8±4.91
B (Negative control)	0.5ml	174.6±2.61[a]	98.8±2.61[a]	35.8±3.70[a]
C (500mg/kg)	0.5ml	159.6±2.19*	78.0±3.74*	28.0±4.18*[a]
D (750mg/kg)	0.5ml	159±2.00*	74.8±2.77*	25.9±4.06*
E (1g/kg)	0.5ml	160.4±1.52*	74.6±1.95*	25.7±3.78*

*Result are expressed in mean ± SEM (n=5); * Significant at P=.05 compared with the negative control;*
[a] Significant at P=.05 compared with the Normal control

In this work, ALT, AST, and ALP activities were used to determine the protective effects of *C. olitorius* on CCl_4 induced hepatotoxicity in wistar rats and were interpreted as follows;

Statistical evaluation revealed that, for AST levels, the extract recorded a significant (P=.05) decrease for all treatment groups; C (159.6±2.19*), D (159±2.00*), E (160.4±1.52*) when compared with negative control B (174.6±2.61). Similar trend were also observed in ALT and ALP levels.

Table 3. Effect of crude extract of *C. olitorius* on serum biochemical parameters of CCl_4 induced hepatotoxicity in wistar rats

Treatment	Dose	Biochemical parameters		
C.olitorius	CCl_4 /kg	Total protein (g/l)	Albumin (g/l)	Billirubin (u/l)
A (Normal control)		49.8±2.59	23.2±2.59	14.72±0.98
B (Negative control)	0.5ml	52.4±1.95	29.6±1.14	18.28±0.97
C (500mg/kg)	0.5ml	60.4±1.95*[a]	26.2±1.92*	19.6±1.73[b]
D (750mg/kg)	0.5ml	61.2±2.86*[a]	25.6±2.41*	20.86±0.34[b]
E (1g/kg)	0.5ml	64.9±3.13*[a]	24.8±3.11*	19.8±0.35[b]

Result are expressed in mean±SEM (n=5); Significant at P=.05 compared with the negative control; [a] Significant at P=.05 compared with the Normal control; [b] Not significant at P=.05 compared with negative control*

For the biochemical parameters in the Table 3, statistical analysis showed that the ethanolic leaf extract of *C. olitorius* recorded a significant (*P*=.05) increase for total proteins level for all treatment groups; C (60.4±1.95), D (61.2±2.86), and E (64.4±3.13) when compared with negative control B (52.4±1.95). There was no significant (*P*=.05) change observed for billirubin in all treatment groups when compared with negative control. However, significant (*P*=.05) decrease was observed for albumin in the *C.olitorius* treated group when compared with those treated with CCl_4 alone.

Table 4. Effect of crude extract of *C. olitorius* on serum hematological parameters of CCl_4 induced hepatotoxicity in wistar rat

Treatment	Dose	Hematological Parameters			
C. olitorius	CCl_4/kg	PCV (%)	Platelet (10^5/µL)	WBC (10^9/l)	Hb (%)
A Normal control		40.50±0.18	2.89 ±0.77	2.60±0.8	12.19±0.17
B Negative control	0.5ml	42.60±0.8	4.40±2.4	3.10±0.7	13.10±1.0
C (500mg/kg)	0.5ml	37.0±2.10	2.61±0.07*	1.73±0.26*	13.40±2.4
D (750mg/kg)	0.5ml	39.0±0.95	2.92±0.41*	1.33±0.31*	12.10±2.0
E (1g/kg)	0.5ml	43.3±0.95*	3.4±0.98*	1.40±0.13*	13.0±1.40

*Result are expressed in mean ± SEM (n=5); [a] Significant at P=.05 compared with the positive control; *Significant at P=.05 compared with the negative control*

For the hematological parameters in Table 4 above, statistical analysis showed that the ethanolic leaf extract of *C. olitorius* recorded no significant (*P*=.05) change for PCV levels for all treatment groups except group E with 1g/kg body weight; C (37.4±2.10), D (39.0±0.95), and E (43.3±0.95) when compared with both normal control A (40.50±0.18) and negative control B (42.60±0.8). However a significant (*P*=.05) decrease was observed for platelet and WBC level when compared to the negative control but Hb was not significant when compared with both normal and negative control.

4. DISCUSSION

The presence of tannins, steroids, saponin, terpenoids, cardiac glycosides and alkaloids as revealed in the results of phytochemical analysis in *C. olitorius* leaf extract suggests its usage for various medicinal purposes in folk medicine. Alkaloids are the most efficient therapeutic plant substance. Both natural and synthetic alkaloids are used as basic medicinal agent because of their analgesic, antispasmodic and antibacterial properties [38,39].

4.1 Effect of Crude Extract of *C. olitorius* on Serum Enzyme Activities of CCl_4 Induced Hepatotoxicity in Wistar Rats

The result showed that CCl_4 caused an elevation in the serum content of ALT, AST, and ALP. This indicates liver injury especially the rise in ALT activity [40,41]; AST and ALT are reliable determinants of liver parenchyma injury [42]. Activities of both ALT and AST significantly increased in CCl_4 treated rats indicating liver dysfunction. Hence, serum or plasma enzymes levels have been used as indices for monitoring chemically induced tissue damages [43]. From the result of this study, it was observed that treatment of Wistar rats with ethanolic leaf extract of *C. olitorius* at different concentration after CCl_4 administration caused a significant reduction in hepatotoxicity. This is evidenced from marked decreased in serum ALT, AST, and ALP activities of those treated with the extract (P=.05) relative to the group treated with CCl_4 alone. This could be that since high level of the enzymes are associated with hepatic injuries; probably this cellular protection could be due to the presence of β-Carotene (a precursor of vitamin) in *C.olitorius* plants [44]. The marked decrease in the activities of these three marker enzymes (ALT, AST and ALP) agrees with the studies carried out by other researchers on CCl_4 hepatotoxicity of other herbal plants; such as *Hibiscus rosasinenis* [45], Rooibios tea (*Aspalathus linearis*) and *Moringa olifera* leaf [43].

4.2 Effects of Crude Extract of *C. olitorius* on Serum Biochemical Parameters of CCl_4 Induced Hepatotoxicity in Wistar Rats

The extract at high doses of 750mg and 1g/kg body weight caused significant increase in total protein levels when compared with both controls, which probably indicates that the buffering capacity of the blood and body fluid balance have been enhanced. Adewuyi and Afolayan (2009) gave similar report [46] in their work on *Pelargonium reniforme* extract treated rats. The extract caused insignificant change in Billirubin levels of the treated rats when compared with both controls, which suggests the maintenance of status quo in plasma level of metals, ions, fatty acids, amino acids, billirubin and enzymes. Adedayo and coworkers [47] also gave a similar report. At all doses of 500mg/kg, 750mg/kg and 1g/kg body weight of the treated rats, the extract caused significant reduction in Albumin levels when compared with the negative control, which could indicate a burst in both the natural and acquired immunity of the body against invading CCl_4.

4.3 Effect of Crude Extract of *C. olitorius* on Serum Hematological Parameters of CCl_4 Induced Hepatotoxicity in Wistar Rats

The values obtained for PCV showed non-significant effects of 15-day treatment of rats with *C. olitorius* on packed cells volume (PCV) when compared with both normal and negative control. This is an indication that there was no destruction of red blood cells and no change in the rate of production of RBC (erythropoiesis).This also shows that *C. olitorius* does not have the potential to stimulate erythropoietin release from the kidneys, which is the humoral regulator of RBC production [48]. The non-significant effects of the treatment of CCl_4 induced rats with *C. olitorius* at all doses also indicate that there were no change in the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since RBC is very important in transferring respiratory gases [49]. The significant (P=.05) reduction in white blood cell count (WBC) on the treated rats when compared with both controls suggest the presence of some bioactive agents likely to cause such destructed or imposed production of WBCs [50,51].Similar report was given by Oyedeji and Bolarinwa

[52]. Also, the treatment of rats with *C. olitorius* at all treatment doses may not have adverse effects on the bone marrow, kidney and hemoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and hemoglobin metabolism [53].

5. CONCLUSION

In conclusion, this study has shown that ethanolic leaf extract of *C.olitorius* could have hepatoprotective property and to large extent biochemically safe; however, due to the decreased level of WBC, it suggest that the extract contains some bioactive agents that could cause such destructed or impairs hematopoietic activities [43,50,51].

6. RECOMMENDATION

Further study on the histopathology should be carried out and the urea, creatinine and other electrolyte levels should be determined to affirm the therapeutic safety of the plant. In addition, the active ingredient (s) should be isolated, purified and used for further research to determine whether the decrease in the level of WBC is as a result of some bioactive agents (like saponins), causing destruction or impairments of hematopoietic activities.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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