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PROTECTIVE AND CURATIVE ACTIVITY OF ETHANOL LEAF EXTRACT OF CORCHORUS OLITORIUS IN THIOACETAMIDE EXPOSED RATS

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ABSTRACT

The present study was undertaken to evaluate the protective and curative effect of ethanol leaf extract of *Corchorus olitorius* against thioacetamide-induced toxicity in experimental rats. The animals were exposed to thioacetamide at a dose of 400 mg/kg body weight i. p for 3 selected days exhibited a significant increase ($p < 0.05$) in hepatic and renal biomarker enzymes namely; ALT, AST, ALP and increase concentration of bilirubin, urea and creatinine. Pre-treatment and post-treatment with extract of *Corchorus olitorius* at dose of 200 mg/kg body weight orally significantly decrease the hepatic and renal marker enzymes and concentration of bilirubin, urea and creatinine. The effects of *Corchorus olitorius* leaf extract on histological features of liver of experimental rats indicated that the normal control, extract pretreated and post-treated showed normal hepatic architecture while positive control group B shows occluded central vein and dilated sinusoids. Histopathological section of the kidney showed normal kidney stroma with Bowman's capsule with glomerulus (renal corpuscle) and renal tubules, in pretreated, post-treated and normal control groups, while thioacetamide treated group showed infiltration of inflammatory cells. Present investigation showed that the ethanol leaf extract of *Corchorus olitorius* have considerable protective and curative effect against thioacetamide-induced hepatic and renal oxidative damages in rats.

KEYWORDS: *Corchorus olitorius*, nephroprotective, hepatoprotective, thioacetamide, creatinine.

INTRODUCTION

Over decades of research, the roles of free radicals-induced organ injury still remain a matter of debate and currently a serious issue in diagnosis and management of many diseases. Free radicals cause cell damage through mechanism of covalent binding and lipid peroxidation leading to injury of the cell membranes.[1] Common free radicals include; superoxide anion, Nitric oxide, Peroxyl, Hydroxyl, Lipid Peroxyl, Alkyl and alkoxyl radicals which are involved in various catalysis of enzymes, transition metals or biological systems.[2] However non-free radicals species such as Hydrogen Peroxide, Singlet Oxygen, Ozone, Lipid Peroxide are other forms of activated oxygen generated from aerobic organisms capable of reacting with most biological molecules including proteins, lipids, lipoprotein and DNA.[3] The reactive ability of free radicals have been considered responsible for a series of undesired processes such as aging, material degradation, food deterioration, oxidative stress and many pathophysiological disorders such as arthritis, diabetes, inflammation, neuro-degeneration and cancer.[2-4] Thioacetamide (C_2H_5NS; TAA), is a thioamide compound that exists at room temperature as colorless to yellow crystals with a slight odour of mercaptans. Currently, thioacetamide is used only as a replacement for hydrogen sulfide in qualitative analyses and as a reactant in making metal salt nanoparticles.[5] The primary routes of potential human exposure to thioacetamide are inhalation and dermal contact. Consumers could have been exposed to thioacetamide residues through contact with products for which it was used as a solvent in the manufacturing process.[6] Thioacetamide (TAA) has long been known as a hepatotoxicant. Its biotransformation to thioacetamide sulfoxide (TAAS) occurs along the cytochrome P-450 (CYP)-dependent pathway, TAAS is subsequently converted to thioacetamide disulfoxide, a toxic reactive metabolite. These reactive metabolites covalently bind to liver macromolecules and dramatically increase the production of reactive oxygen species which then induce acute centrilobular liver necrosis.[1] Interestingly, antioxidants have been found to terminate or reduce oxidative process by scavenging free radicals and as protective agents in humans, reduce cellular oxidative damage and thus retard the progress of many chronic diseases as well as lipid peroxidation.[7] However nature remains the primary sources for cure to many ailments and diseases as antioxidants of natural source have been identified in many medicinal plants which have raised various interests in this field.[8] Numerous indigenous vegetables in Africa were consumed innocently without information on

their dietary and health benefits.[9] Most suspected bioactive components of these plants are believed to have important roles with natural antioxidant properties which are presumed to be safe since they occur in plants hence the need for laboratory based experiments and investigations to validate these claims which form the rationale for this study. *Corchorus olitorius*, commonly known as Nalta jute, tossa jute, Jew's mallow, West African sorrel and bush okra, is a species of shrub in the family Malvaceae which is the primary source of jute fibre.[10] *Corchorus olitorius* leaves are the leaves of certain jute plants, used as a food source in Asia, the Middle East, and parts of Africa.[11] It is a popular vegetable in West Africa. The Yoruba tribe of Nigeria calls it "ewedu" while the Hausas and Fulbe neighbours call it "ram".[12] The leaves are rich in betacarotene, iron, calcium, and Vitamin C. The plant has an antioxidant activity with a significant α-tocopherol equivalent of Vitamin E. Vitamins A, C and E present in jute leaf/Saluyot "spongeup" free radicals, scooping them up before they can commit cellular sabotage.[11,13] *Corchorus olitorius* leaf as vegetable contains an abundance of antioxidants that have been associated with protection from chronic diseases such as heart disease, cancer, diabetes, and hypertension as well as other medical conditions.[14] The present study was aimed at evaluating the protective and curative effect of ethanol leaf extract of *Corchorus olitorius* in thioacetamide-induced rats.

MATERIALS AND METHODS

Chemicals/ Reagents

The AST, ALT, ALP, Urea, Creatinine and Bilirubin kits were purchased from Randox Laboratories limited, 55 Diamond Road, county Antrim, BT29 4QY, United Kingdom. All other reagents / chemicals obtained from standard supplies were of analytical grade.

Animals

Twenty-four male wistar albino rats weighing 170-200g were bought from the Department of Pharmacology Animal House, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University. The animals were housed in standard cage and acclimatized for one week. Animals were maintained at the temperature of 25 ± 2^{0}c, 45 ± 5% relative humidity and 12h light/dark cycle and had access to water and food freely.

Preparation of Ethanol Leaf Extract of *Corchorus Olitorious*

The leaf of *Corchorus olitorius* was used for this study. The plant was identified and confirmed by a botanist Prof. Ajibeshin Kolawole of Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Nigeria. The leaves were cleaned and made free from

sand and shade dried for eight days. The dried leaves were ground into powder using a blending machine. 280grams of the powder was soaked in 3litres of ethanol (95% v/v) which serve as solvent in a plastic bucket and stirred in every 4hours for 24hours before filtering. The filtrate was added into beakers and evaporated to dryness for 37^0 C for 72hours. Appropriate weight (17g) of the residue was prepared in normal saline to obtain various concentrations of the extract that were administered orally to the rats.

Experimental Design

The male wistar albino rats were divided into four groups comprising of six animals each.

Group A: **(Normal control):** Receive normal saline water.

Group B (Positive control): Administered 400mg/kg of thioacetamide (i. p) on the 1^{st}, 5^{th}, 10^{th} day of study.

Group C (Pretreated): Administered 200mg/kg extract of *Corchorus olitorius* orally for 2ldays + 400mg/kg thioacetamide (i. p) on the 11^{th}, 15^{th} and 20^{th} day of study.

Group D (Post treated): 400mg/kg thioacetamide (i. p) on the 1^{st}, 5^{th} and 10^{th} +200mg/kg extract of *Corchorus olitorius* orally from 11^{th} day to 20^{th} day of study.

Animals were fasted for 24hrs and sacrificed on the 22^{nd} day of study. Blood was collected into plain bottles for various biochemical analyses. The liver and kidney were harvested and fixed in 10% formalin for histological study.

Collection of Samples

Biochemical Analysis

Animals were sacrificed on the 22^{nd} day of study, by neck decapitation and blood samples were collected from the animals through cardiac puncture into plain bottles and allowed to clot and centrifuged at 3000rpm for 15 minutes. The resultant sera were used for biochemical analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Urea, Creatinine and Bilirubin according to appropriate standardized procedures using commercially available kits. The liver and kidney were excised using a midline abdominal incision, weighed and transferred into 10% neutral buffered formalin for histopathological examination.

Biochemical Assay

Serum transaminase (ALT and AST) was determined by method of,[15] ALP by Phenolphthalein monophosphate method.[16] Bilirubin was estimated by colorimetric method.[17] Serum Urea was estimated by[18] method and serum creatinine by.[19]

Statistical Analysis

All data were expressed as Mean ± Standard deviation. The data obtained were analyzed using Two-way Analysis of Variance (ANOVA) using SPSS (Statistical Package for Social Sciences) Version 20. The means were separated and compared by post-Hoc and Turkey method. $P < 0.05$ was considered as statistical significant.

RESULTS

Table 1 shows the effect of ethanol leaf extract of *Corchorus olitorius* on AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), bilirubin, urea and creatinine in thioacetamide exposed rats. It shows that the activities of AST, ALT, ALP and the concentration of bilirubin, urea and creatinine in rats administered with thioacetamide (positive control) were significantly increased ($p<0.05$) compared to the normal control. However, the extract (pretreated and post-treated groups) significantly decrease the activity of AST, ALT, ALP and the concentration of bilirubin, urea and creatinine compared to the positive control.

The results also indicated significant decrease in the mean body weight of thioacetamide treated rats compared with the normal control. However, *Corchorus olitorius* leaf extracts significantly increase ($p<0.05$) the mean body weight difference in both pretreated and post-treated groups compared with the positive control. The mean weight of liver and kidney increase significantly ($p<0.05$) in extract pretreated and post-treated groups compared with thioacetamide exposed group. (Table 2).

Figure 1 shows the effects of *Corchorus olitorius* leaf extract on histological features of liver of experimental rats. Histological features of the normal control, groups C and D showed normal hepatic architecture while positive control group B shows occluded central vein and dilated sinusoids.

Histopathological section of the kidney showed normal kidney stroma with Bowman's capsule with glomerulus (renal corpuscle) and renal tubules, in pretreated, post-treated and

normal control groups, while thioacetamide treated group showed infiltration of inflammatory cells. (Fig. 2).

Table 1: Effect of *Corchorus olitorius* on thioacetamide-induced Liver and Kidney injury in rats.

	Group A	Group B	Group C (pretreated)	Group D (post treated)
	Normal Control	Positive Control (TAA)	200mk/kg Extract + 400mg/kg of TAA	200mk/kg Extract +400mg/kg of TAA
AST (U/L)	29.5±6.5[a]	43.5±8.3[b]	38.5±9.5[c]	32.5±9.5[a]
ALT (U/L)	32.0±6.8[a]	51.5±9.3[b]	40.0±8.8[c]	34.0±10.1[a]
ALP (U/L)	282±3.31[a]	358±15.2[b]	332±13.4[c]	299±4.1[a]
BIL (µmol/L)	1.32±1.01[a]	2.11±0.9[b]	1.99±0.9[c]	1.41±0.7[a]
Urea (Mmol/L)	55.20±0.26[a]	69.31±2.19[b]	65.04±2.12[c]	58.01±3.52[a]
Creatinine (mg/dl)	7.92±0.32[a]	9.91±0.71[b]	8.57±0.53[a]	8.73±0.51[a]

Values are represented as Mean ± SD, n=6. Value with different superscripts (on the rows) from control are statistically different at $p<0.05$.

Effect of *Corchorus olitorius* on Mean Weights of Liver and Kidyney In thioacetamide-induced Liver and Kidney injury in albino rats

Groups	Mean wt (g) rats day1	Mean wt(g) rats day 21	Mean wt(g) difference	Mean wt(g) rats liver	Mean wt(g) rats kidney
G1 (Normal Control)	173.3±8.3	199±7.1	26.0±1.2[a]	5.38±2.1[a]	1.64±6.3[a]
G2 (Positive Control)	180±28.4	178±21.3	-2.0±7.1[b]	4.33±2.8[b]	1.31±2.1[b]
G3 200mk/kg Extract +400mk/kg of TAA (Pretreated)	173.3±11.3	178±10.3	5.0±1.0[c]	5.93±7.2[c]	1.44±2.8[c]
G4 200mg/kg Extract +400mg/kg of TAA (Post-treated)	170±10.5	185±13.5	15.0± 3.0[d]	6.96±6.3[d]	1.48±7.2[d]

Values are represented as Mean ± SD, n=6. Value with different superscripts from control are statistically different at $p<0.05$.

Haematoxylin and Eosin, X100

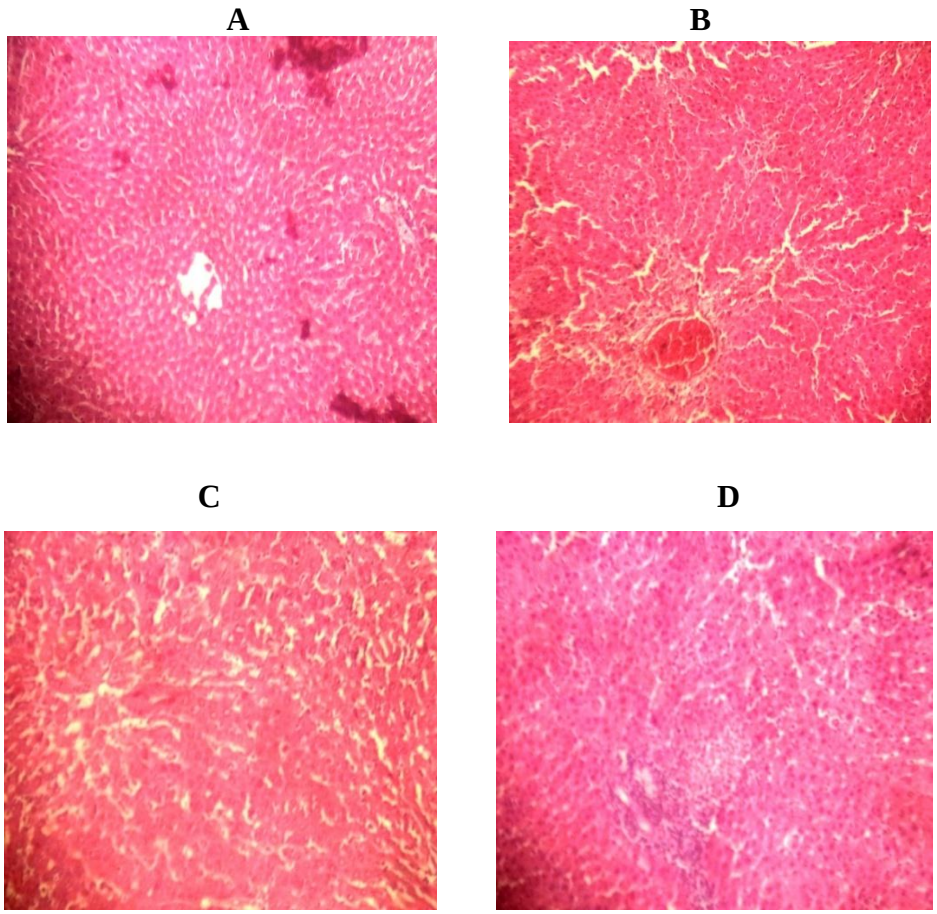
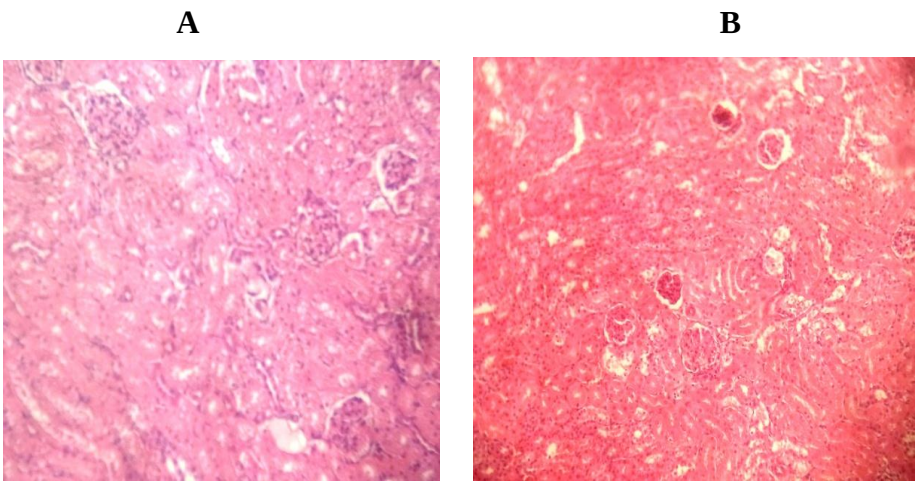
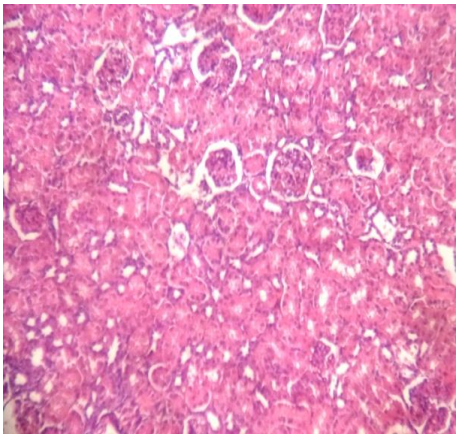
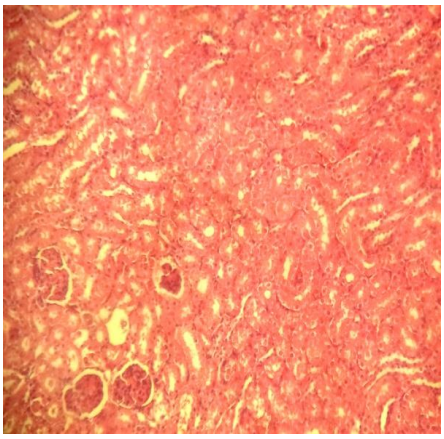


Fig. 1: Histopathological images of liver pretreated with ethanol extract of *Corchorus olitorius* in thioacetamide exposed albino rats. A: Normal control, B: Positive control, C: Pretreated group, D: Post-treated group.

Haematoxylin and Eosin, X100.







C **D**

Fig. 2: Histopathological images of kidney pretreated with ethanol extract of *Corchorus olitorius* in thioacetamide exposed albino rats. A: Normal control, B: Positive control, C: Pretreated group, D: Post-treated group.

DISCUSSION

The present study evaluated the protective and curative effects of ethanol leaf extract of *Corchorus olitorius* in thioacetamide-induced albino rats. Considerable evidence indicates that increased oxidative damage is associated with and may contribute to the development of all major hepato and nepho-degenerative diseases.[20] Thioacetamide is a model hepatotoxicant, consumed to induce acute and chronic liver injury due to its effects on protein synthesis. Thioacetamide undergoes bioactivation to sulfene, a reactive metabolite that is responsible for the release of factors directing to centrilobular necrosis, protein denaturation, lipid peroxidation and impairment of urea cycle.[21] We observed that thioacetamide intoxication caused injury in the liver and kidney of rats by disturbing the prooxidant/antioxidant status as revealed from the altered levels of different serum liver and kidney biochemical indices. Pretreatment and post-treatment with extract of Chorchorius olitorius could prevent the toxin-induced oxidative impairment of selected tissues in thioacetamide intoxicated rats. Herbs and other plant products are found to be rich in polyphenolic compounds with free radical scavenging and protective antioxidant defenses in the body,[22] due to their natural source.

Thioacetamide intoxication caused increase in serum level of AST, ALT, ALP and the concentration of bilirubin. Elevated levels of these serum enzymes have been studied and could be indicative of an early liver damage as well as an assessment of its functions,[23,24]

However results obtained showed that rats pretreated and post-treated with extract ameliorated the effects as these parameters were restored nearly to their control levels. This then suggest the possible modulatory role of the extract for possible hepatoprotective ability,[25]

Corchorus olitorius leaves are rich in betacarotene, iron, calcium, and Vitamin C. The plant has an antioxidant activity with a significant α-tocopherol equivalent of Vitamin E. Vitamins A, C and E present in jute leaf/Saluyot "sponge up" free radicals, scooping them up before they can commit cellular sabotage,[11,13]

Corchorus olitorius leaf as vegetable contains an abundance of antioxidants that have been associated with protection from chronic diseases such as heart disease, cancer, diabetes, and hypertension as well as other medical conditions.[14]

Thioacetamide intoxicated rats, showed elevated levels of Urea and Creatinine, which are important nephrotic biomarkers when compared with the normal control. Severe liver disease with destruction of cells leading to impairment of the urea cycle resulting to diminish glomerular filtration, urea retention and decrease in urea excretion is seen in renal disease.[26] Creatinine retention in the blood is evidence of kidney impairment.

This study, indicated that extract of *Corchorus olitorius* (pretreated and post-treated groups) at 200mg/kg significantly decrease the level of urea and creatinine ($P<0.05$), when compared with the thioacetamide control.

The presence of antioxidant constituents such as betacarotene, Vitamin C, Vitamin E and Vitamin A might be responsible for the hepatoprotectivity and nephroprotectivity of the extract as well its curative activity. This is also in agreement with,[27] who reported that leaves extract of *Corchorus olitorius* have considerable protective effect against sodium arsenite-induced hepatic and renal oxidative damages.[28] later reported that the extract of *Corchorus olitorius* were hepatoprotective, had antioxidative and antilipiperoxidative effect on rats exposed to sodium arsenite.

The effects of *Corchorus olitorius* leaf extract on histological features of liver of experimental rats indicated that the normal control, extract pretreated and post-treated showed normal hepatic architecture while positive control group B shows occluded central vein and dilated sinusoids.

Histopathological section of the kidney showed normal kidney stroma with Bowman's capsule with glomerulus (renal corpuscle) and renal tubules, in pretreated, post-treated and normal control groups, while thioacetamide treated group showed infiltration of inflammatory cells.

Present investigation showed that the ethanol leaf extract of *Corchorus olitorius* have considerable protective and curative effect against thioacetamide-induced hepatic and renal oxidative damages in rats.

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