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Hepatoprotective Activity of Methanol Tuber Extract of *Cyperus esculentus* Linn on CCL_4-Induced Hepatotoxicity in Albino Rats (*Rattus norvegicus*)

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Abstract
This study investigated the hepatoprotective properties of methanol tuber extract of *Cyperus esculentus* on carbon tetrachloride (CCL_4)-induced hepatotoxicity in albino rats. Fresh tubers of *C. esculentus* were collected, dried under shade, ground into powder, and extracted by cold maceration technique using 80% methanol. The extract obtained was filtered, dried, and subjected to phytochemical analysis. Thirty six (36) adult male albino rats were randomly assigned to 6 groups (A–F) of 6 rats each. Sub-acute hepatotoxicity was induced in groups A–E by intra-peritoneal (i/p) injection of 1 mg/kg body weight (bw) of CCL_4 in equal volume of olive oil at 3 days interval for 12 days. Group A was given 10 ml/kg distilled water as placebo and served as untreated control, groups B, C, and D were treated with 200, 400 and 800 mg/kg *Cyperus esculentus* methanol tuber extract (CEME) respectively, group E was treated with 100 mg/kg silymarin as positive control, while group F was given 10 ml/kg distilled water as placebo and served as normal control. Treatment with CEME and silymarin was carried out for 15 days. Assay of serum enzymes and other bio-chemicals was done at the end of the 15-day experimental period following standard procedures. Treatment with CEME and silymarin significantly ($p<0.05$) reduced serum ALT, AST, ALP, bilirubin, and liver weight percentage of body weight, and also led to slight elevation of serum total protein of the albino rats whose liver were damaged using CCL_4. These findings strongly suggest that CEME significantly protected the hepatocellular integrity, ameliorated impaired hepatic excretory function and slightly improved hepatic synthetic ability in experimentally induced sub-acute liver damage.

Keywords: Hepatotoxicity, tiger nut, *Cyperus esculentus,* methanol tuber extract, albino rats.

Introduction

The liver is amongst the most complex and important organs in the human body. It plays a major role in metabolism and detoxification of drugs and xenobiotics (Anthea *et al*, 1993). It is constantly and variedly exposed to xenobiotics which may lead to hepatotoxicity (Saukkonem *et al.,* 2006). Liver diseases are amongst the major health problems in the world today, and remain a global health challenge despite notable development in modern medicine (Williams, 2006). *Cyperus esculentus* Linn commonly known as tiger nut is an emergent grass-like plant belonging to the family *Cyperaceae* (De Vries, 1991). It is a freely growing tuber, which is widely consumed in its natural form (Ejoh *et al*., 2006). It is rich in energy, soluble glucose and oleic acid, vitamins and minerals (Temple *et al*., 1990; Omode *et al*., 1995), and also possesses relatively high total antioxidant capacity because it contains considerable amounts of water-soluble flavonoids and glycosides (Eteshola and Oraedu, 1996).

The tubers of *C. esculentus* are under-utilized due to lack of information on their nutritional benefits and medicinal potential, hence the present study which evaluated the hepatoprotective potential of methanol tuber extract of *C. esculentus* in experimentally-induced liver damage in albino rats.

Materials and Methods

Fresh yellow tubers of *Cyperus esculentus* were bought from Nsukka market in Enugu State, Nigeria, in December 2016. The tubers were identified by a plant taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. They were washed clean and allowed to dry under shade, and afterwards ground into powder. One thousand grammes (1000g) of the powdered tubers were extracted with 80% methanol using the cold maceration technique. The resultant extract was concentrated to dryness and referred to as *Cyperus esculentus* methanol tuber extract (CEME). Phytochemical analysis was carried out on the extract following standard procedure (Harborne, 1998). Forty eight (48) adult male albino rats (*Rattus norvegicus*) of 12 weeks of age obtained from the Laboratory Animal Unit of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, were used for the study. They were housed in stainless steel cages in a fly proof animal house at room temperature, and allowed 2 weeks to acclimatize before

the commencement of the study. They were fed commercial pelletized feed (Grand Cereals Nig. Ltd, Jos, Nigeria), and provided with clean water *ad libitum* all through the study. Twelve of the albino rats were used for acute toxicity test (OECD, 2001).

Thirty six albino rats were randomly assigned to six groups (A - F) of six rats each. Sub-acute hepatotoxicity was induced in rats in groups A – E by intra-peritoneal (i/p) injection of 1 ml/kg CCL_4 in equal volume of olive oil at the beginning of the experiment (day 0), and after every 72 hours for 12 days. Group A served as untreated control, groups B, C and D were treated with 200, 400 and 800 mg/kg CEME respectively, group E was treated with silymarin (a standard hepatoprotective drug) at the dose of 100 mg/kg as positive control, while group F was treated with 10 ml/kg of distilled water as placebo and served as normal control. Treatment with CEME and silymarin was done *per os* twice daily for 15 days. Blood samples were collected from the albino rats on day 15 for serum biochemistry assay. Blood sample collection was by the orbital technique, while the serum biochemistry assay was done immediately upon separation of the serum from blood clot, following standard procedures (Colville, 2002) using Quimica Clinica Applicada (QCA) test kits (QCA, Spain). The serum enzymes and bio-chemicals evaluated were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol and bilirubin, total proteins, albumin and globulin. The rats were humanely sacrificed afterwards on the 15th day by intra-peritoneal injection of 200 mg/kg of thiopentone sodium (AVMA, 2013). Gross changes in the liver were observed, and relative liver weight of body weight of the individual rats was determined. Guidelines for the use of animals for laboratory experiment were strictly adhered to (NRC, 1985).

Data obtained from the study were subjected to one way analysis of variance (ANOVA), and variant means were separated post hoc using the least significant difference (LSD) method. Significance was accepted at $p<0.05$.

Results and Discussion

Results of the acute toxicity test in table 1 showed that the albino rats tolerated the extract up to 5000mg/kg, therefore the LD_{50} of the extracts is above 5000 mg/kg thereby placing CEME on 'Category 5 – Unclassified' of the Global Harmonized Classification System (OECD 2001). This implies that CEME is not acutely toxic, and is thus considered safe for acute use as treatment of ailments and diseases for which it is effective.

Table 1: Effects of oral administration of CEME on serum enzymes activity, cholesterol and bilirubin levels of rats given sub-acute toxic doses of CCL_4. [TChol. = Total Cholesterol; TBil. = Total bilirubin]

	Means ± standard error				
*Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TChol. (mg/dl)	TBil. (mg/dl)
Group A	130.22±30.87 [a]	177.23±34.39 [a]	262.64±16.22 [a]	89.54±10.42	2.58±0.10 [a]
Group B	71.33±26.55 [b]	89.38±9.95 [b]	225.59±10.64 [ab]	86.42±7.51	2.24±0.03 [b]
Group C	73.57±18.71 [b]	111.31±7.59 [b]	228.82±15.95 [ab]	81.95±5.18	2.36±0.12 [ab]
Group D	51.25±7.18 [b]	96.44±8.47 [b]	192.02±32.51 [ab]	84.54±6.96	2.17±0.10 [b]
Group E	58.49 ±10.41[b]	110.17±11.59 [b]	182.30±20.79 [b]	87.38±5.35	2.29±0.12 [ab]
Group F	30.13±2.00 [b]	54.09±1.10 [c]	187.70±23.68 [b]	73.60±5.07	1.46±0.06 [c]

Alphabetical superscripts in a column indicate significant differences between the groups, $p<0.05$. * Group treatments: Group A – CCL_4 + distilled water placebo; Group B - CCL_4 + 200mg/kg bw of CEME; Group C - CCL_4 + 400mg/kg bw of CEME; Group D - CCL_4 + 800mg/kg bw of CEME; Group E = CCL_4 + 100mg/kg bw Silymarin; Group F - Distilled water placebo only.

The phytochemical analysis showed that CEME has high levels (+++) of carbohydrates, glycosides, fats and oil; and moderate levels (++) of tannins, flavonoids and saponins. This result is in agreement with the reports of Chukwuma *et al*. (2010), and suggests that CEME may possess the natural antioxidants that are necessary for protection against free radical damage by CCL_4 in the liver. Temple *et al* (1990) reported that tiger nuts have a relatively high total antioxidant capacity because they contain considerable amount of water-soluble flavonoids and glycosides which scavenge free radicals *in-vivo.*

The serum ALT, AST and ALP activity levels of rats in groups B, C, D, E and F were significantly ($p<0.05$) lower than those of group A (Table 1). The administration of CEME was protective of the hepatocellular integrity of the liver of rats whose liver were damaged with CCL_4, and compared effectively with silymarin (Table

1). The comparatively higher serum enzyme activity of ALT, AST and ALP in groups A, B, C, D and E rats is an indicator of the ability of CCL_4 to induce liver damage by disrupting hepatocellular integrity and liver function (Kim *et al*., 2010). The ability of administered CEME to protect hepatocellular integrity as recorded in this study agrees with the reports of Farok *et al* (2011) who also recorded significant decreases in ALT, AST and ALP in rats pretreated with aqueous extract of *Cyperus esculentus* tuber. The protective activity of the extract on hepatocellular integrity may be due to the presence of phytochemicals like flavonoids and water soluble glycosides in the extract, as CCL_4 -induced hepatotoxicity is known to be produced mainly by oxidative stress (Muriel *et al*., 2001).

There were no significant variations in the serum cholesterol level amongst the groups, but the serum total bilirubin level of group A rats was significantly ($p<0.05$) higher than that of groups B, D, and F (Table 2). This is suggestive that CEME at these doses enhanced hepatic clearance of bilirubin. Bilirubin is the ultimate breakdown product of hemoglobin and serves as a diagnostic marker of liver and blood disorders. When the liver cells are damaged, they may not be able to excrete bilirubin in the normal way, thus causing a build-up of bilirubin in the blood and extracellular fluid (Singh *et al*., 2011).

The serum total protein level of rats in group E and F was significantly higher ($p<0.05$) than that of group A, while the serum albumin of only group F rats was significantly higher ($p<0.05$) than group A (Table 2). There were no significant variations in the serum globulin level amongst the groups (Table 2). The depletion of serum total protein and albumin in rats that were given CCl_4 is a further indication of liver dysfunction associated with CCl_4 administration (Navarro and Senior, 2006). Treatment with CEME led to slight elevation of serum total protein and albumin of the treated rats. The liver weight percentage of body weight of rats in groups A and B was significantly ($p<0.05$) higher than that of rats in groups C, D, E and F (Table 2). This is an indication of inflammation which accompanies hepatotoxicity (Bukhsh, 2014). Treatment with the extract (groups C and D) and silymarin (group E) significantly reduced this inflammation induced by CCL_4 toxicity.

Table 2. Effects of oral administration of CEME on serum proteins and liver weight percentage of body weight of rats given sub-acute toxic doses of CCL_4.

*Groups	Means ± standard error			
	Total proteins (g/dl)	Albumins (g /dl)	Globulins (g/dl)	Liver weight % of bw
Group A	5.87 ± 0.20 ^{a}	2.92 ± 0.30 ^{a}	2.95 ± 0.33	4.23 ±0.14^{a}
Group B	5.95 ± 0.31 ^{a}	3.31 ± 0.29 ^{ab}	2.64 ± 0.23	4.05 ± 0.14^{ab}
Group C	6.37 ± 0.30 ^{ab}	3.60 ± 0.20 ^{ab}	2.77 ± 0.20	3.70 ± 0.15^{b}
Group D	6.47 ± 0.06 ^{ab}	3.45 ± 0.16 ^{ab}	3.01 ± 0.17	3.78 ± 0.05^{b}
Group E	6.79 ± 0.36 ^{b}	3.53 ± 0.27 ^{ab}	3.26 ± 0.22	3.74 ± 0.13^{b}
Group F	6.73 ± 0.24 ^{b}	3.72 ± 0.15 ^{b}	3.02 ± 0.12	3.31 ± 0.09^{c}

Alphabetical superscripts in a column indicate significant differences between the groups, $p<0.05$. * Group treatments: Group A - CCL_4 + distilled water placebo; Group B - CCL_4 + 200mg/kg bw of CEME; Group C - CCL_4 + 400mg/kg bw of CEME; Group D - CCL_4 + 800mg/kg bw of CEME; Group E = CCL_4 + 100 mg/kg bw Silymarin; Group F - Distilled water placebo only.

Conclusion

The results of this study strongly suggest that the methanol tuber extract of *Cyperus esculentus* can be safely used for the treatment of sub-acute toxic liver injury.

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