

# RESEARCH AND REVIEWS: JOURNAL OF PHARMACOLOGY AND TOXICOLOGICAL STUDIES

## Hepato-protective Activities of Tiger Nut (*Cyperus esculentus*) Against Hepatotoxicity Induced by Carbon Tetrachloride in Rats.

Oyedepo TA<sup>1\*</sup>, and Odoje OF<sup>2</sup>.

<sup>1</sup>Biochemistry Programme, Department of Basic Sciences, Adeleke University, Ede, Nigeria.

<sup>2</sup>Department of Chemistry, Emmanuel Alayande College of Education, Oyo, Nigeria.

### Research Article

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#### \*For Correspondence

Biochemistry Programme,  
Department of Basic  
Sciences, Adeleke University,  
Ede, Nigeria.

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#### ABSTRACT

Tiger nuts (*Cyperus esculentus*) are under-utilized due to lack of information on their nutritional potential. A lot of people eat the tiger nut without knowing the nutritional benefits and products that can be obtained from it. Tiger nut milk is used as a liver tonic in China and some other countries. The purpose of this study therefore was to investigate the protective effects of Tiger nut against a dose of Carbon-Tetrachloride (CC1<sub>4</sub>) induced liver damage in experimental rats. The induction of liver damage was done by intraperitoneal administration of CC1<sub>4</sub> (0.5ml/kg body weight in olive oil). This led to significant increases in the levels of L-aspartate aminotransferase (AST), L-alanine transferase (ALT), alkaline phosphatase (ALP) and lipid peroxides in the CC1<sub>4</sub> intoxicated rats. Pretreatment with varied concentrations of Tiger nut diets (1%, 5% and 10%) and vitamin E (9 %,) for 21 days prior to CC1<sub>4</sub> administration resulted in significant decreases in liver marker enzymes and lipid peroxides. The results of this study therefore suggest that Tiger nut may be acting as a natural antioxidant that prevents hepatic oxidative stress induced by CC1<sub>4</sub>.

#### INTRODUCTION

Tiger nut (*Cyperus esculentus*) is an emergent grass- like plant belonging to the sedge family <sup>[1]</sup>. It is widely distributed in the temperature zones within South Europe as its probable origin, and has become naturalized in Ghana, Nigeria and Sierra Leone <sup>[2]</sup>. Tiger nuts have long been recognized for their health benefits as they have a high content of soluble glucose and oleic acid, along with high energy content (starch, fats, sugars and proteins). They are rich in minerals such as phosphorous and potassium, calcium, magnesium and iron necessary for bones, tissue repair, muscles, the blood stream and for body growth and development and rich in vitamins E and C <sup>[3, 4]</sup>.

There are mainly three varieties namely: black, brown and yellow, and only yellow and brown are readily available in the Nigerian markets. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size, attractive colour and fleshier body. The yellow variety also yields more milk, contains lower fat and higher protein and less anti-nutritional factors especially polyphenols <sup>[5]</sup>. Sugar-free tiger nut milk is suitable for diabetic people and also helps in weight control <sup>[6]</sup>, due to its content of carbohydrates with a base of sucrose and starch (without glucose), and its high content of Arginine, which liberates the hormone that produces insulin <sup>[7]</sup>. It is recommended for those who suffer from indigestion, flatulence and diarrhoea because it provides digestive enzymes like the catalase, lipase and amylase <sup>[5]</sup>. The high content of oleic acid has positive effect on cholesterol, thereby preventing heart attacks, thrombosis and activates blood content of soluble glucose. Tiger nut reduces the risk of colon cancer. It prevents constipation. Tiger nut contains a good quantity of vitamin B<sub>1</sub>, which assists in

balancing the central nervous system and helps to encourage the body to adapt to stress [8]. The milk supplies the body with enough quantity of Vitamin E, essential for fertility in both men and women.

In China, tiger nut milk is used as a liver tonic, heart stimulant, drunk to heal serious stomach pain, to promote normal menstruation, to heal mouth and gum ulcers, used in Ayurvedic medicines and is a powerful aphrodisiac (sexual stimulant). The black species of the tiger nut is an excellent medicine for breast lumps and cancer. The tubers have a relatively high total antioxidant capacity, because they contain considerable amounts of water-soluble flavonoid glycosides [9].

In Nigeria, tiger nut is available in fresh, semi-dried and dried form in the markets where it is sold locally and consumed even uncooked. These nuts are under-utilized due to lack of information on their nutritional potential [10]. A lot of people eat the tiger nut without knowing the nutritional benefits and products that can be obtained from it like tiger nut oil and milk. The purpose of this study was to investigate the hepatoprotective potential of the tiger nut that is completely unexploited in Nigeria.

## MATERIALS AND METHODS

Tiger nuts (*Cyperus esculentus*) purchased from local market in Oyo, Oyo State, Nigeria were sliced and dried in the air for 5 days. The dried, sliced seeds were ground into flour with an electric blender (Model MX – 795N-National)

Male albino rats of the Wistar strain (150g-180g) were obtained from the University of Ibadan animal house. Animals were maintained under standard environmental condition (28-30°C, 60-70 % relative humidity, 12-h dark / light cycle) in stainless steel cages with free access to standard laboratory animal diet (Vital finisher) and drinking water. They were left to acclimatize to laboratory conditions for 7 days prior to commencement of the experiment.

### Experimental Procedure

The rats were divided into six groups with each group comprising five animals. Rats in groups 1 and 2 received the pelleted diet and water, while those in groups 3, 4 and 5 were fed with diet formulated with the flour of *Cyperus esculentus* and rat pellets as follows:

Group 3: 1% flour of *Cyperus esculentus*;  
Group 4: 5% flour of *Cyperus esculentus*  
Group 5: 10% flour of *Cyperus esculentus*

Also, animals in group 6 received diet compounded with vitamin E and rat pellets (9% vitamin E).

All the rats in the various groups received their respective diets and water ad libitum for 21 days. On the 22nd day of the experiment, CCl<sub>4</sub> (0.5ml/kg body weight in 0.5 olive oil) was administered intraperitoneally to rats in groups 2, 3, 4, 5 and 6. The animals were allowed to fast for 24 hours after which they were anaesthetized in a chloroform saturated chamber [11].

Blood samples were obtained by cardiac puncture from each rat by means of a 5ml hypodermic syringe and needle. The blood samples were introduced into clean, dry bottles without anticoagulants for serum separation. The bottles and its contents were centrifuged at 5000g for 10 minutes.

Serum was collected into a clean, dry sample container. The serum levels of L-aspartate aminotransferase (AST), L-alanine transferase (ALT) and alkaline phosphatase (ALP) were measured spectrophotometrically as described by Verly [12]. The liver was excised, washed in ice-cold saline, and homogenized at 0.1 M Tris-HCl buffer (pH 7.4; 40C) in a homogenizer at 600 rpm for 4 minutes using mortar and pestle [13]. The liver homogenate was employed in assaying the activities of the lipid peroxides as described by Hunter *et. al.* [14] and modified by Gutteridge and Wilkins [15].

The mean values of the various groups were compared using analysis of variance (ANOVA) and the level of significance was set at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

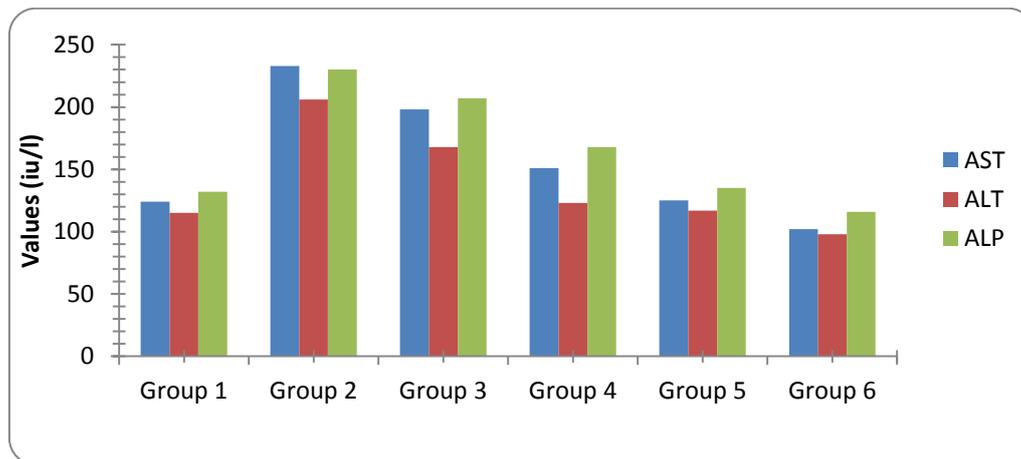
The effects of pretreatment with Tiger nut and vitamin E, 21 days prior to CCl<sub>4</sub> administration on liver enzymes and lipid peroxides in rats is shown in Table 1, figures 1 and 2.

Rats in Group 2 that received a single dose of CCl<sub>4</sub> showed marked elevation of the liver enzymes when compared with those of the group 1 (control) rats. However, the pretreated groups 3, 4, 5 and 6 rats showed significant decline in the levels of AST, ALT and ALP when compared with group 2 rats that received CCl<sub>4</sub> alone.

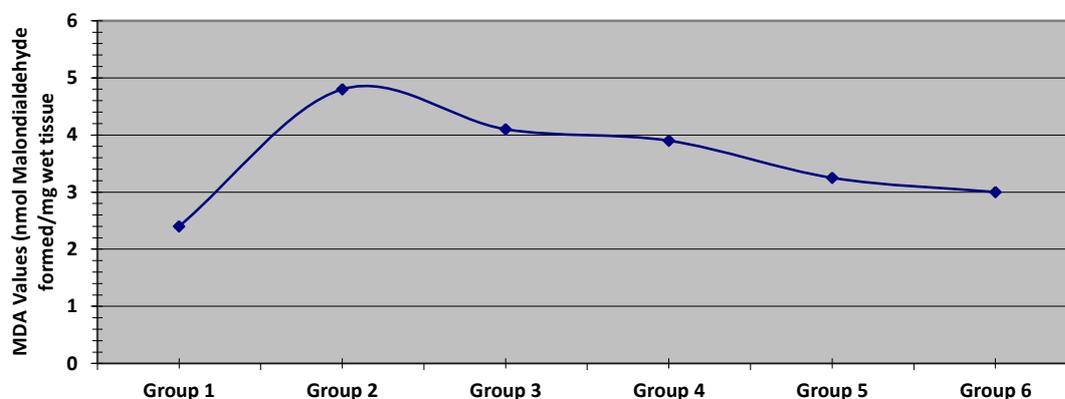
**Table 1: Effects of Varied Levels of *Cyperus esculentus* and Vitamin E on Liver Enzymes of Rats Administered CCl<sub>4</sub>**

Animal Group	Level of Liver Enzymes (iu/l)		
	AST	ALT	ALP
Group 1	124.50±2.75 <sup>a</sup>	115.80 ± 3.35 <sup>a</sup>	132.55 ± 3.60 <sup>a</sup>
Group 2	233.75 ± 3.70 <sup>b</sup>	206.65 ± 4.45 <sup>b</sup>	132.35 ± 2.75 <sup>b</sup>
Group 3	198.80 ± 2.25 <sup>c</sup>	168.25 ± 2.70 <sup>c</sup>	207.30 ± 1.20 <sup>c</sup>
Group 4	151.75 ± 1.80 <sup>d</sup>	123.55 ± 2.40 <sup>d</sup>	168.20 ± 2.20 <sup>d</sup>
Group 5	125.55 ± 2.80 <sup>e</sup>	117.45 ± 3.30 <sup>e</sup>	135.50 ± 1.70 <sup>e</sup>
Group 6	102.25 ± 1.25 <sup>a</sup>	98.80 ± 1.20 <sup>a</sup>	116.60 ± 2.60 <sup>a</sup>

Values are expressed as mean ± STD; n=5 in each group. Values of enzymes with different letters (a, b, c, d, e) in the respective groups are significantly different at P≤0.05.



**Figure 1: Effects of varied levels of *Cyperus esculentus* and Vitamin E on liver enzymes of rats administered CCl<sub>4</sub> (mean ± STD; n=5 in each group).**



**Figure 2: Activities of lipid peroxides in rats pretreated with *Cyperus esculentus* seed and Vitamin E prior to CCl<sub>4</sub> administration.**

Increased levels of lipid peroxides were recorded in the liver tissue of group 2 rats as shown in figure 2. The activities of the peroxides decreased with increase in the amount of the *Cyperus esculentus* in the feed formula. This is confirmed in the values obtained in rats in groups 3, 4, and 5. Similarly, rats in group 6 recorded malondialdehyde values close to those of group 5 and 1 (control) rats.

Administration of CCl<sub>4</sub> in this study resulted in a significant hepatic damage as indicated by the elevation in the levels of liver marker enzymes (AST, ALT, ALP). This indicated that CCl<sub>4</sub> intoxication compromised the integrity of the hepatic cell membranes [16]. The elevated level of the enzymes obtained in this study validates those of Nishigaki, et. al. [17], Raja, et. al. [18] and Shafaq et. al. [19] who reported elevated levels in the serum content of hepatic enzymes in rats administered with CCl<sub>4</sub>.

The evaluation of the preventive action in liver damage induced by CCl<sub>4</sub> has been widely used for hepatoprotective drug screening. CCl<sub>4</sub> is a widely used experimental hepatotoxicant which requires metabolic activation by the liver cytochrome P-450 enzymes to form highly reactive toxic metabolites such as trichloromethyl radical (CCl<sub>3</sub>·) and peroxy trichloromethyl radical (CCl<sub>3</sub>OO·). Both trichloromethyl and its peroxy radical (CCl<sub>3</sub>OO·) are capable of covalently binding to proteins or lipids of cell membranes and organelles, abstracting hydrogen atoms from polyunsaturated fatty acid (PUFA), initiating lipid peroxidation thus causing damage to cell membrane, disturbing Ca<sup>2+</sup> homeostasis, changing enzyme activities, and finally inducing hepatic injury or necrosis [20,21].

Since the above mechanism is suggestive of the process of oxidative stress, it is true, therefore, that any natural product with antioxidant property will prevent or reverse lipid peroxidation; including cell membrane damage. Previous study by Temple et al. [4] that tiger nuts have a relatively high total antioxidant capacity, because they contain considerable amounts of water-soluble flavonoid glycosides and the fact that tiger nut milk is used as liver tonic in China prompted this study.

The results of this study show that pretreatment of rats 21 days preceding CCl<sub>4</sub> administration caused a marked decline in the levels of hepatospecific serum enzymes. This suggests that Tiger nuts (*Cyperus esculentus*) may be protective against CCl<sub>4</sub>-induced liver damage in rats. This was established by a comparative analysis of the results obtained in rats pretreated with *Cyperus esculentus* and vitamin E.

Malodialdehyde (MDA) is a product of lipid peroxidation [22]. An increase in the liver MDA levels is a signal of elevated level of lipid peroxidation [23]. Extensive lipid peroxidation leads to disorganization of membrane by peroxidation of unsaturated fatty acids which also alters the ratio of poly-unsaturated to other fatty acids. This would lead to a decrease in the membrane fluidity and the death of cells.

## CONCLUSION

The results of the present study indicate that Tiger nuts (*Cyperus esculentus*) exhibited a potential hepatoprotective activity against carbon tetrachloride induced hepatotoxicity and validate the traditional use of this nut as liver tonic. The significant decrease in the levels of lipid peroxides recorded in rats pretreated with *Cyperus esculentus* suggests that the nuts may possess the natural antioxidants necessary for protection against free radical damage induced by CCl<sub>4</sub> in rat liver. The Tiger nut, though under-utilized, is therefore still a good food snack for all. Further studies are required to isolate and characterize the active principles, which are responsible for the hepatoprotective efficacy of this valuable medicinal seed.

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