Assess The Potential Protective Effect of Germinated Tiger Nuts on Some Biochemical and Histopathological Markers in Hepatotoxicity Rats

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ABSTRACT

A current study was undertaken to evaluate the potential hepatoprotective effect of germinated tiger nuts (GTNs) on the betterment of some biochemical and histopathological markers in hepatotoxicity rats. Fifty rats (200±7g) were exploited and haphazardly separated into five groups (n=7). Group 1 was employed as a negative control group (normal rats). Group 2 (positive control group) was injected intraperitoneal (IP) with CCl_4 at a dose of 2 ml/kg of b. wt. once/week for 6 weeks and fed on the basal diet lone. The remaining three groups (3, 4 and 5) were IP with 2 ml/kg of b.wt. of CCL_4 once/week (6 weeks) and fed on the prepared diet with GTNs at levels of 5, 10 and 15% respectively. In comparison with the normal rats, positive rats have a significant decrease in body weight (BW), relative body weight gain (RBWG%) and feed consumed (FC), as well as activity of CAT, SOD and GPx enzymes in liver tissues. There is a significant increase in serum levels of AST, ALT, ALP, albumin, Cr, UN, UA, total lipids, TG and TC, as well as MDA level in liver tissues. In comparison with the positive group, injecting rats with CCl4 and feeding the complemented diet with the different proportions of GTNs (5, 10 and 15%) had a significant improvement in BWG, RBWG, FC and FER, as well as the tested parameters in serum and liver tissues. Histological investigation of the liver showed a remarkable advancement in being detected by normal hepatocytes and slight hepatic steatosis, especially in treated

rats with 10 and 15% of GTNs. Eventually, GTNs might mitigate the failure of liver and kidney functions and improve the lipid profile and antioxidant defense due to its antioxidant ingredients, therefore eliminating the deleterious effect of some toxic agents like CCl_4.
Keywords: Tiger Nuts; Germination; Hepatotoxicity; Liver Functions; Kidney Functions; Histopathology, CCl_4; Oxidative Stress.

1. INTRODUCTION

The liver plays a major role in the metabolism and removal of non-drugs or drug detoxification (**Pandit *et al.*, 2012**). The liver is undergoing deterioration and incapability from several factors, such as environmental toxicants, medicine, and microbial metabolites, which are the main causes of liver disease and death worldwide (**Upadhyay *et al.*, 2010**). Age, gender, lifestyle, fatness, nutritional states, genetic factors, doses and durations of drugs used are the most factors that promote susceptibility of a person to a potentially hepatotoxic (**Boelsterli *et al.*, 2006**). There are further than a thousand medications and chemicals that have been having been formally to cause liver damage (**Porceddu *et al.*, 2012**). Medicament-induced liver injury is a relatively prevalent cause of acute liver disease and carries mortality (**Björnsson *et al.*, 2013**).

The tiger nut, also known as the underground nut, is the teeny tuber (*Cyperus Esculentus* L.,) and grows all over the world due to its higher crop and global utilization (**Rubert *et al.*, 2011**). Although there are three types of tiger nuts according to its color (black, brown and yellow), the yellow type is prioritized over all other types due to its properties like its bigger size, attractive color and fleshier body **(Adebayo and Arinola, 2017)**. In the Egyptian local market, the brown and yellow colors are the most abundant types and are eaten after soaking as a kind of nut because of their sweet taste.

Tiger nuts are distinguished by their higher content of nutrients such as lipids, starch, fibers and ashes (**Adel *et al.*, 2015**), and bioactive substances such as organic acids, alkaloids and phenols (**Nina *et al.*, 2019**). It also contains tannins and saponins, which have anti-inflammatory and anti-bacterial properties (**Vega-Moralesa *et al.*, 2019**), good source of minerals (phosphorus, potassium, calcium, magnesium, and iron) and vitamins (E, C, and B1) (**Maduka and Ire,**

2018). Tiger nuts are used in a wide range of food applications, such as snacks, beverages (**Sánchez-Zapata *et al*., 2012**) and gluten-free bread due to their nutritive value and sweety taste (**Aguilar *et al*., 2014**). Although the content of protein is relatively small in tiger nuts, it is established to be suitable for diabetic patients or those with digestive dysfunctions and may prevent heart disease (**Ogunlade *et al*., 2015**). The dietary fiber in this tuber is effective in the prohibition of colon cancer, obesity and gastrointestinal disorders (**Viuda-Martos *et al*., 2010**). Due to the existence of flavonoids, the tiger nut can be used as a good source of natural antioxidants (**Jing *et al*., 2015**).

The tiger nut is consumed as a healthful plant because of its multiple vitamins, minerals, as well as arginine, which are helpful in the release of the hormone output of insulin (**Bamishaiye and Bamishaiye, 2011**). Moreover, the tiger nut has a beneficial effect on body development, tissue repair, muscles, the bloodstream and bones mineralization due to its abundance of necessary minerals (**Mohdaly, 2019**). Also, it has a preventative effect against gastrointestinal disorders, obesity and diabetics (**Achoribo and Ong, 2017**), maintained the immunological system and tissue preservation (**Roselló-Soto *et al*., 2019**).

Tiger nut is a commodity source of edible oils that contains an abundance of monounsaturated fatty acids, especially, oleic acid, and it is similar to olive oil in its nutritional value (**Roselló-Soto *et al*., 2018**). Also, oil of tiger nut has lower polyun-saturated fatty acid and acidity, which makes it excellent for the skin (**Mohdaly, 2019**). In addition, it possesses antioxidant, antiarthritic, antiinflammatory, antispasmodic, antibacterial and analgesic (**Krichène *et al*., 2016**).

The natural process of germination that occurs throughout the period of seed growth and meets the minimum conditions for growth ameliorates calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid content (**Kaushik *et al*., 2010**) as well increases protein, minerals, and vitamin concentration, and reduces tannin and phytic acid content in seeds (**Adebayo and Arinola, 2017**).

In Egypt, several of the available traditional natural plants have attracted researchers due to their possibility and effectiveness against drug-induced liver toxicity. Therefore, the existing study was managed to estimate the potential effectiveness of germinated tiger

nuts (GTNs) on the improvement rate of some biochemical and histopathological markers in hepatotoxicity rats.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Tiger Nuts Tuber: Dried tiger nut tubers (**Photo 1**) were obtained from Harraz Comp., for Agriculture., Seeds, Herbs and Medicinal Plants, Cairo, Egypt.

2.1.2. Rats and Diet: Adult Albino rats of Sprague Dowley strain weighing 200 ± 7gm were obtained from Laboratory Animal Colony, Ministry of Health and Population, Egypt. Rats were housed in wire cages at the animal house at the Home Economics Faculty, Helwan Univ. and were given food and water *ad libitum*. All constituents of the basal diet (casein, cellulose, choline chloride, D-L methionine and constituents of vitamins and minerals) were purchased from the El-Gomhouria Comp. for Trad. Drugs and Chemicals, Cairo, Egypt. Sucrose, corn starch and soybean oil were purchased from the local market in Cairo, Egypt.

2.1.3. Chemicals and Kits for Biochemical Analysis: Carbon tetrachloride (CCL_4) of 100 % concentration and all other used chemicals were purchased from the El-Gomhouria Comp. for Trad. Drugs and Chemicals, Cairo, Egypt. Kits for the biochemical analysis of total lipids, total glycerides and total cholesterol, liver functions (AST, ALT, ALP and Alb), kidney functions (Cr, BUN and UA), as well as malondialdehyde (MDA) and antioxidant enzymes includes CAT, SOD and GP-x were purchased from the Sigma-Aldrich Chemie GmbH, Egypt.

2.2. Methods

2.2.1. Preparation and Germination of Tiger Nuts Tuber: Dried tiger nut tubers (DTN) were cleaned, sorted from broken tubers and other foreign materials, and washed with tap water to remove dust. Cleaned tubers were soaked in tap water overnight (12hr.) at room temperature. Thereafter, the tubers were separated from soaking water (**Photo 2**) and covered in strainer by wet covering cotton cloth

and left to germinate for 96 hr at room temperature with frequent watering as described by **Amany *et al.*, (2014)**. After the accomplishment of the germination process (**Photo 3**), the germinated tiger nuts (GTNs) were dried using a drying oven vacuum at 50-55 C°. A grinder mill and sieves were used to obtain a powder particle size of less than 0.4mm. The final flour was packaged and stored until further use.

Photo 1:
Dried Tiger Nut

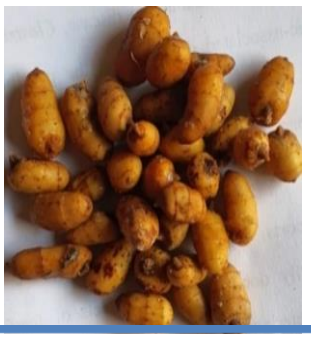


Photo 2:
Soaking Tiger Nut

Photo 3:
Germinated Tiger Nut

2.2.2. Preparation of The Basal Diet and Formulated Diets with Tiger Nut Powder: The normal AIN- 93 M basal diet was formulated based on the approbated quantities of **Reeves *et al.*, (1993)** to meet the nutritional requirements for rats during the experimental period. A complementary diet was prepared by adding tiger nut powder at 5, 10 and 15% of the basal diet.

2.2.3. Doses and Route of Administration for Induction of Hepatic Injury: During the experimental period (6 weeks), all groups of rats, except the negative control group (normal rats), were injected intraperitoneally (IP) once a week with 2 ml/kg CCl_4 dissolved in olive oil of a ratio 1:1 (V:V) to induce hepatic cell injury according to the described methods by **Sundraresan and Subramanian (2003)**. Normal rats were injected IP with similar doses of olive oil alone.

2.2.4. Experimental Design and Grouping of Rats: Fifty rats weighing 200 ± 7gm were randomly divided into five groups, each

of ten animals. All groups of rats were housed in a healthy environment at room temperature (22±2°C), 12/12 hr. of the light/dark cycle, humidity of 45 to 50%, and water and food were provided ad libitum. Afterwards and prior to the experimental study, all groups were kept for one-week acclimatization.

After an acclimatization period, normal rats were injected IP with olive oil alone. The other three groups were administrated IP by CCl_4 at a dose of 2ml/kg of b. wt. dissolved in olive oil (1:1) once a week during the experimental period. Then, each group was named according to the type and level of treatment as follows:

Group 1(- ve): Normal group (negative control group) fed on the basal diet alone.

Group 2 (+ ve): Untreated hepatotoxicity rats (positive control group) fed on the basal diet alone.

Group 3 (Treated): Hepatotoxicity rats fed on the accompanied diet with germinated tiger nuts (GTNs) at a level of 5%.

Group 4 (Treated): Hepatotoxicity rats fed on the accompanied diet with GTNs at a level of 10%.

Group 5 (Treated): Hepatotoxicity rats fed on the accompanied diet with GTNs at a level of 15%.

2.2.5. Estimation of Food Intake, Body weight Gain and Relative Body Weight Gain: The total amount of feed consumed (FC) per day for each rat was calculated depending on the total amount consumed daily for each group. The change in body weight was determined by weighing the rats at the initial of the experimental period (IBW) and at the end of the experimental period (FBW). In that case, body weight gain (BWG) and relative body weight gain (RBWG%) were estimated using the following equations, respectively, as outlined by **Kratochvílova *et al.*, (2002)**.

BWG = FBW (g) - IBW (g)

RBWG% = FBW (g) - IBW (g) / IBW (g) X 100

2.2.6. Blood Collecting and Liver Sampling Preparation: At the end of the experimental interval (six weeks), all rats were forbidden to eat, except for water for about 12 hr. Afterwards, each animal was anaestheticized with diethyl ether and sacrificed. Heart puncture was done using a 5 ml syringe for drawing of blood into clean dried centrifugation tubes. Subsequently the collected blood samples were

permitted to clot at room temperature. Subsequently, serum was separated by using centrifugation adapted at 3000 rpm for 15 min. Then, clear serum samples were taken into the closed Eppendorf pipe and preserved in the deep freeze at -20°C until they were used for biochemical analysis.

The liver of each rat was carefully removed, washed with normal saline for blood removal, and one gram of each liver was taken and frozen. Subsequently, frozen liver tissues (1g) were washed with ice-cooled NaCl solution (0.9%), and homogenized in 100 ml solution of ice-cooled potassium chloride (1.5%) with 50 mmol solutions of potassium phosphate buffer (pH 7.4) to produce 1% homogenate solution (W/V). Then, homogenate tissues were centrifuged at 4000 rpm for 10 min at 4°C, and the supernatant was separated and stored until used in the biochemical analysis.

2.2.7. Biochemical analysis

Estimation of Liver Function: The serum activity of liver enzymes (AST, ALT and ALP) and concentration of Albumin were measured calorimetric by utilizing Elabscience® ELISA Kits according to the methods of **Young (1997)** and **Friedman and Young (1997)**. The spectrophotometer was adapted at 520 nm, 570 nm, 405 nm and 620nm, respectively.

Estimation of Kidney Functions: The Kidneys function was inspected through the quantitative determination of serum levels of creatinine (Cr), urea nitrogen (UN) and uric acid (UA) in all rats using calorimetric instructions of QuantiChrom™ Assay Kits as described by **Waiker and Bonventre (2008)**, **Lum and Leal-Khouri (1989)** and **Young (2000)** respectively. The spectrophotometer was adapted at 570nm, 520 nm and 590nm, respectively**.**

Estimation of Lipids Profile: Serum levels of total lipids (TL), triglycerides (TG) and total cholesterol were determined spectrophotomically according to EnzyChrom ™ Assay Kits instruction manual at 540nm, 570nm and 570nm, individually, as mentioned by **Lutzke and Brauler (1990), Zhu *et al.*, (2000)** and **Admundson and** Zhou **(1999)**, respectively.

Estimation of Malondialdehyde Level and Activity of Antioxidant Enzymes in Liver Tissues: The concentration of MDA, and activity of CAT, SOD and GSH-Px enzymes in the obtained supernatants of liver tissues homogenated were colorimetrically determined using commercial Elabscience assay Kits. Optimum wavelengths for determination were 532 nm, 405 nm, 450 nm and 412 nm, respectively, as mentioned by **Gaschler and Stockwell (2017)**, **Glorieux and Calderon (2017)**, **Cristiana *et al*., (2014)** and **Chu *et al*., (1993)**.

2.2.8. Liver Histopathological Inspection: The procedures for histopathological inspection of the liver of each rat were performed in line with the referred procedure by **Bancroft and Gamble (2002)**. Concisely, clean liver samples were dipped in buffered formalin (10%) for about one week. Thereafter, the fixed specimens were desiccated in graded ethanolic alcohol from 50 to 100%. Then, specimens were cleared by Xylol, and fixed and deeped in paraffin bulk, then segmented in thickness from 4 to 6 microns and coloured with the Heamtoxylin and Eosin stain for examination.

2.2.9. Statistical analysis: All the obtained data of biochemical analysis were analyzed statistically by one-way of variance (ANOVA) using computerized SPSS package program (SPSS 20.00 software for Windows) and expressed as Mean ± Standard Error (SE). Significant differences among means was estimated at $p<0.05$.

3. RESULTS

The effect of the supplemented basal diet with the different levels (5, 10 and 15%) of GTNs on FBW, BWG, RBWG (%) and FC in CCl_4-induced liver toxicity rats are instituted in Table **1**. Our results discovered that the treated rats with CCl_4 alone (positive control rats) had a significant ($p<0.05$) decrease in FBW, BWG, RBWG (%) and FC, compared to the normal control rats. Feeding rats on an accompanied basal diet with the three different levels of GTNs combined with IP injection by CCl_4 caused a significant ($p<0.05$) increase in FBW, BWG and RBWG (%) in comparison to injecting rats with CCl_4 alone.

More suitable results in FBW, BWG and RBWG (%) were found in treated groups with fed on a supplemented diet by 15% of GTNs, compared to the normal and positive control groups. While, there is a significant increase and no significant change, as well as a significant decrease in FC in feeding hepatotoxic rats on complementing basal diet with, 5, 10 and 15%, respectively, compared to positive rats.

Table 1: The effect of complementing basal diet by GTNs on BWG, RBWG and FC in hepatotoxicity rats.

Groups / Parameters	-ve	+ve	Treated groups with GTNs at levels of:		
			5%	10%	15%
IBW (g)	204.29±0.42^{b}	205.86±0.34^{a}	202.43±0.43^{c}	202.14±0.51^{c}	203.86±0.51^{b}
FBW (g)	266.14±0.34^{b}	234.57±0.48^{e}	243.14±0.40^{d}	260.00±0.49^{c}	271.29±0.36^{a}
BWG (g)	61.85±0.02^{b}	28.71±0.02^{e}	40.71±0.05^{d}	57.68±0.03^{c}	67.43±0.02^{a}
RBWG (%)	30.28±0.24^{b}	13.95±0.21^{e}	20.12±0.43^{d}	28.63±0.49^{c}	33.08±0.41^{a}
FC (g/d)	15.71±0.10^{a}	15.21±0.10^{b}	15.71±0.10^{a}	15.21±0.10^{b}	14.71±0.10^{c}

Values are expressed as Mean ± Standard Error (M±SE), Means with different letters in each row are significantly differs at p< 0.05; **- ve**: Negative Control Group; **+ve**: Positive Control Group; **GTNs**: Germinated Tiger Nuts. **IBW**: Initial body weight; **FBW**: Final Body Weight; **BWG**: Body Weight Gain; **RBWG**: Relative Body Weight Gain; **FC**: Food Consumed;

The results in Table **2** exemplify the effectiveness of nourishing rats on the supplemented diet with GTNs at the levels of 5, 10 and 15%, respectively, on the serum activity of AST, ALT, ALP enzymes and concentrations of albumin (Alb) in normal and hepatotoxic (untreated and treated) rats. Tabulated results have proven that CCl_4 induced liver injury as detected by the significant ($P<0.05$) increase in serum activities of AST, ALT and ALP enzymes and the decrease in serum concentration of Alb, compared to the negative control group.

In contrast, treating rats by feeding them on the complimentary diet with GTNs at the different levels co-incorporated with CCl_4 caused a significant ($p<0.05$) reduction in serum activities of AST, ALT and ALP enzymes, and increased Alb concentrations, compared with the positive group. The optimum results were shown in the treated hepatotoxic rats by feeding them on the complementing diet with GTNs at a level of 15% along with IP injected by CCl_4, compared to that treated with GTNs at levels of 5 and 10%, and positive rats.

Results in Table **3** exhibit the effect of feeding on the complementing diet with GTNs on serum levels of Cr, UN and UA in rats with hepatotoxicity. The results showed a significant increase in serum Cr, UN and UA concentrations in rats treated with CCl_4 alone (positive control group), compared to the negative control group. On the other hand, there was a significant deficiency in serum Cr, UN and UA levels of the CCl_4-treated groups with the different levels of GTNs in combination, compared to the treated rats with CCl_4 alone.

Table 2: The effect of complementing basal diet by GTNs on serum activity of AST, ALT, ALP enzymes and concentrations Alb in hepatotoxicity rats.

Groups / Parameters	-ve	+ve	Treated groups with GTNs at levels of:		
			5%	10%	15%
AST (u/ml)	43.74±0.26^{e}	95.42±0.21^{a}	82.52±0.37^{b}	70.53±0.39^{c}	53.76±0.38^{d}
ALT (u/ml)	32.89±0.47^{e}	84.26±0.25^{a}	70.38±0.33^{b}	61.32±0.41^{c}	46.79±0.39^{d}
ALP (u/ml)	157.85±0.26^{e}	191.02±0.31^{a}	174.00±0.39^{b}	164.96±0.22^{c}	159.64±0.25^{d}
Alb (µg/ml)	13.44±0.26^{a}	5.88±0.07^{e}	7.00±0.19^{d}	8.36±0.10^{c}	9.77±0.13^{b}

Values are expressed as Mean ± Standard Error (M±SE), Means with different letters in each row are significantly differs at p< 0.05; **- ve**: Negative Control Group; **+ve**: Positive Control Group; **GTNs**: Germinated Tiger Nuts; **AST**: Aspartate aminotransferase; **ALT**: Alanine aminotransferase; **ALP***: alkaline phosphatase; Alb:* Albumin

Table 3: The effect of complementing basal diet by GTNs on serum levels of Cr, UN and UA in hepatotoxicity rats.

Groups / Parameters	-ve	+ve	Treated groups with GTN at levels of:		
			5%	10%	15%
Cr (mg/dl)	2.06±0.09^{e}	8.64±0.12^{a}	7.11±0.08^{b}	5.66±0.12^{c}	3.36±0.08^{d}
UN (nmol/ml)	1.59±0.01^{e}	6.27±0.04^{a}	4.48±0.08^{b}	3.64±0.08^{c}	2.31±0.10^{d}
Uric acid (mg/dl)	1.51±0.03^{e}	5.89±0.07^{a}	3.80±0.09^{b}	2.85±0.06^{c}	1.89±0.06^{d}

Values are expressed as Mean ± Standard Error (M±SE), Means with different letters in each row are significantly differs at p< 0.05; **- ve**: Negative Control Group; **+ve**: Positive Control Group; **GTNs**: Germinated Tiger Nuts; **Cr**: Creatinine; **UN:** Urea Nitrogen; **UA**: Uric acid.

The delimited results in Table **4** summarized that untreated hepatotoxicity rats have a significant ($p < 0.05$) increase in the serum concentrations of TL, TG and TC, as comparative to that of the normal rats. Alongside, feeding rats on a supplemented diet with 5, 10 and 15% of GTNs combined with IP injection by CCL_4 brought about a significant ($p<0.05$) decrease in the serum TL, TG and TC levels, in comparison to treated rats with CCl_4 alone. The most get better in the serum lipid profile tests results were observed in rats treated with the highest level (15%) of GTNs, followed by those treated with 10%.

Table 4: The effect of complementing basal diet by GTNs on serum levels of TL, TG and TC in hepatotoxicity rats.

Groups / Parameters	-ve	+ve	Treated groups with GTNs at levels of:		
			5%	10%	15%
TL (mg/dl)	208.23±1.38^{c}	374.26±0.77^{a}	307.43±2.05^{b}	208.26±1.54^{c}	208.24±0.85^{c}
TG (mg/dl)	83.77±2.92^{e}	159.93±1.9^{a}	124.97±1.30^{b}	104.77±1.73^{c}	89.80±0.85^{d}
TC (mg/dl)	78.53±0.80^{d}	100.53±1.11^{a}	90.50±0.90^{b}	79.57±1.7^{c}	78.54±0.73^{d}

Values are expressed as Mean ± Standard Error (M±SE), Means with different letters in each row are significantly differs at $p< 0.05$; **- ve**: Negative Control Group; **+ve**: Positive Control Group; **GTNs**: Germinated Tiger Nuts; **TL**: Total Lipids; **TG:** Triglycerides; **TC:** Total Cholesterol.

Table **5** represents the effect of a supplemented diet with GTNs on MDA concentrations and activities of CAT, SOD and GSH-Px enzymes in the liver tissues of normal rats, injected rats with CCl_4. In comparison to normal rats, administration of CCl_4 alone stimulates a significant ($p<0.05$) increase in MDA concentration and decreases the activity of CAT, SOD and GSH-Px enzymes in liver tissues. In contrast, feeding rats on the fortified diet with the different levels of GTNs along with IP injection by CCl_4 caused a significant amelioration in MDA concentrations and activities of CAT, SOD and GSH-Px enzymes in liver tissues when compared to IP injected rats by CCl_4 and fed on the normal diet alone.

The preferable improvements in the decrease in MDA concentrations and the increase in the activity of antioxidant enzymes in liver tissues were shown in the treated rats with upper levels (15%) of GTNs.

Table 5: The effect of complementing basal diet by GTNs on concentrations of MDA and activities of CAT, SOD and GPx enzymes in liver tissues of hepatotoxicity rats.

Groups / Parameters	-ve	+ve	Treated groups with GTNs at levels of:		
			5%	10%	15%
MDA (n.mol/mg/protein)	0.80±0.01^{e}	4.55±0.10^{a}	3.05±0.06^{b}	2.01±0.08^{c}	1.06±0.09^{d}
CAT (u/mg/protein)	4.49±0.07^{a}	1.16±0.07^{e}	1.84±0.07^{d}	2.94±0.03^{c}	4.09±0.07^{b}
SOD (u/mg/protein)	3.82±0.08^{a}	0.91±0.01^{e}	1.71±0.07^{d}	2.46±0.12^{c}	3.01±0.03^{b}
GSH-Px (nmol/mg/protein)	2.83±0.10^{a}	0.66±0.04^{e}	0.98±0.02^{d}	1.96±0.05^{c}	2.14±0.04^{b}

Values are expressed as Mean ± Standard Error (M±SE), Means with different letters in each row are significantly differs at $p< 0.05$; **- ve**: Negative Control Group; **+ve**: Positive Control Group; **GTNs**: Germinated Tiger Nuts; **MDA**: Malondialdehyde; **CAT**: Catalase; **SOD:** Superoxide Dismutase; **GSH-Px**: Glutathione peroxidase.

Histopathological Inspection Results:

Microscopic inspections of liver sections of normal rats (negative control group) showed a normal anatomy with no histological abnormalities in a typical central vein (CV), hepatocytes (H) arrangement, the hepatic strands that travel from the label's edge to the central vein sinusoids (S) and portal region as shown in **photo 4**. In contrast, liver sections from untreated rats with hepatotoxicity (positive control group) noticed several marked tissue alterations characterized by hydropic deteriorating cells, altered globular shape and nuclear degradation in some areas, disarrayment of normal hepatic cells, necrosis, severe fatty degeneration, and chromatin condensation (pyknosis). Additionally, the hepatic central vein was discovered to be enlarged and congested, lymphocyte infiltration in the portal area, hepatocytes with cytoplasmic vacuoles swell, and degrade severely (**Photo 5 and 6**). Liver sections from rats fed on the fortified diet with 5% of GTNs combined with IP injection by CCl4 have moderate congestion and fatty degeneration as well as hepatic cells appearing to have pyknotized (dark stained nuclei) and a lot of hydropic degeneration (**Photo7** and **8**). Also, liver sections from rats fed on the fortified diet with 10% of GTNs combined with IP

injection by CCl4 showed evidence of improvement as marked in hepatocytes with active euchromatic nuclei and portal components which appeared to be in good condition. Additionally, the congestion is not severe and there is moderate fatty degeneration with considerable mononuclear cell invasion, and pyknosis (**Photo 9 and 10**). However, the partial improvement in liver tissue was detected in rats fed on a supplemented diet with 15 % of GTNs co-combined with CCl4 by IP injection that exhibited pronounced a few hepatic cells with pyknosis/necrotic changes, as well as mild fatty degeneration (**Photo11 and 12**).

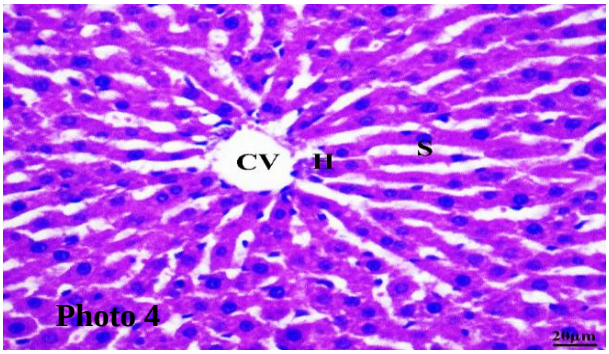
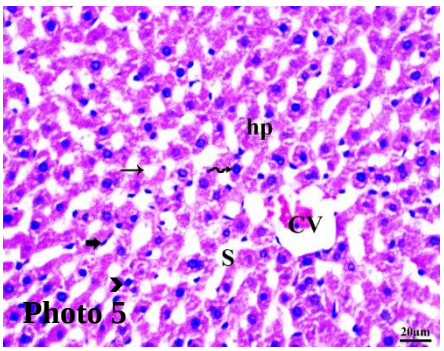


Photo 4: Photomicrograph of liver sections from normal rats (negative control group) showing no histological changes in a typical central vein (CV), hepatocytes (H) arrangement, the hepatic strands that travel from the label's edge to the central vein sinusoids (S) and portal region (H&E).



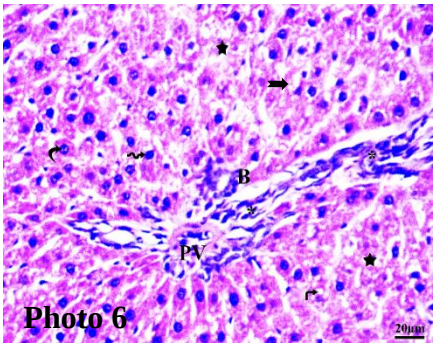


Photo 5 and 6: Photomicrograph of liver section from hepatotoxicity rats (positive control group) showing disarrayment of normal hepatic cells (hp), necrosis, severe fatty degeneration (**thin arrow**), enlarged and congested in hepatic central vein (CV), lymphocyte infiltration in the

portal area (**zigzag arrow**), hepatocytes with cytoplasmic vacuoles swell (**curved arrow**), hydropic deteriorating cells (**bifid arrow**), altered globular shape and nuclear degradation in some areas (**bold star**), chromatin condensation (pyknosis) (**wavy arrow**), binucleated cell (**head arrow**), kupffer cell (**bold arrow**) and karyolysis (**turn arrow**) (H&E).

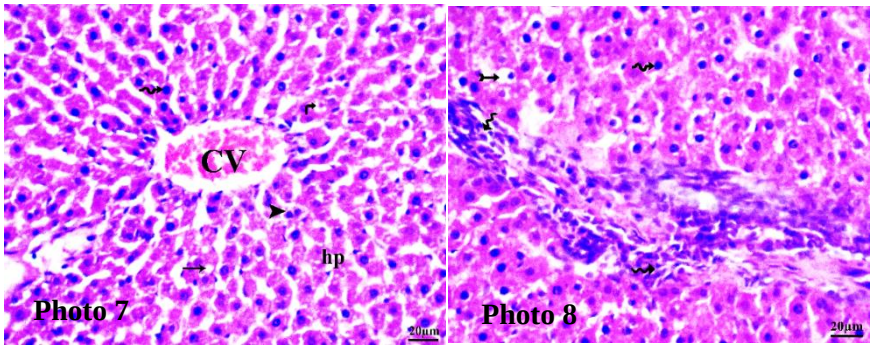


Photo 7 and 8: Photomicrograph of liver section from rats treated by CCl4+ 5% of GTNs showing moderate degenerated hepatocytes (hp), fatty degeneration (**thin arrow**), pyknotized (**wavy arrow**) binucleated cell (**head arrow**) and a lot of hydropic degeneration (**bifid arrow**) (H&E).

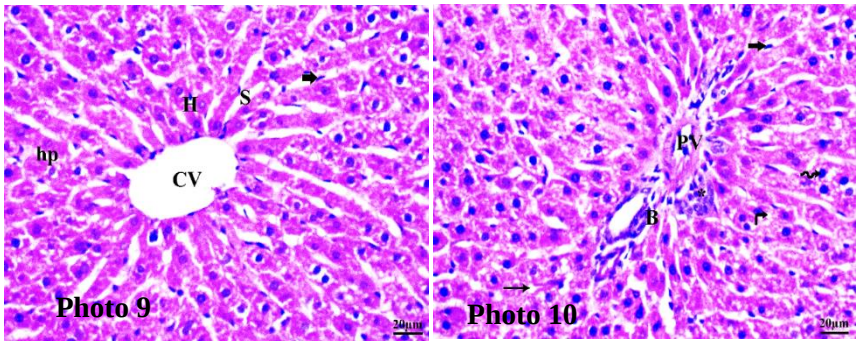


Photo 9 and 10: Photomicrograph of liver section from rats treated by CCl4+ 10% of GTNs showing improvement in hepatocytes with active euchromatic nuclei and portal components which appeared to be normally, there is moderate fatty degeneration (**thin arrow**) with considerable mononuclear cell invasion, and pyknosis (**wavy arrow**) (H&E).

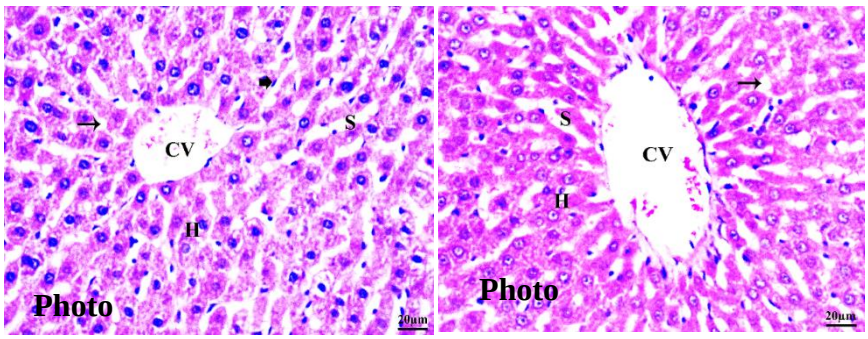


Photo 11 and 12: Photomicrograph of liver section from rats treated by CCl4+ 15% of GTNs showing a few hepatic cells with pyknosis/necrotic changes (wavy arrow) and mild fatty degeneration (thin arrow) (H&E).

H: Hepatic cell or hepatocyte**; S:** Sinusoid**; CV:** Central Vein**; PV:** portal vein**; B=** Bile duct

4. DISCUSIONS

CCL_4 is a toxic substance widely used to induce a hepatotoxic effect in model experimental animals in order to screen for the hepatoprotective effects of some nutritional elements. Although the liver is not the only selective organ as the target of an attack of CCl_4, it likewise affects several vital organs such as the kidney, heart, lungs, brain and testes (**Ozturk *et al.*, 2003**). Also, **Kim *et al.*, (2010)** referred to the fact that CCL_4 is more famous for hepatic injury activity due to its free radical generation. The existing study managed to appraise the potential effectiveness of germinated tiger nuts (GTNs) on the improvement rate of some biochemical and histopathological markers in hepatotoxicity rats caused by CCl_4.

The acquired results exhibit that the IP injection of CCl_4 alone at a dose of 2 ml/kg of b.wt. once a week during the experimental period (6 weeks) caused severe hepatic deterioration, compared to normal rats. This was demonstrable by the significant decrease in BW, RBWG% and FC, as well as activity of CAT, SOD and GPx enzymes in liver tissues. Also, there was a significant increase in serum levels of AST, ALT, ALP, Alb, Cr, UN, UA, TL, TG and TC, as well as MDA levels in liver tissues. Histological inspection discovered severe damage in liver tissue including hydropic cells, altered globular shape, nuclear degradation, and necrosis, severe fatty

degeneration and pyknosis in hepatic cells, as well as the enlarged and congested hepatic and central vein cytoplasmic vacuoles and lymphocyte infiltration in the portal. Our results were consistent with the results of **Peng *et al.*, (2009)** who proved a significant decline in the feed intake (FI), daily body weight gain (BWG) and feed efficiency of the CCl_4 -treated rats, as compared with the control group. Likewise, **Behboodi *et al.*, (2017)** discovered that CCl_4 significantly decreased FI and BWG. The achieved results also concurred, with **Patrick-Iwuanyanwu and Wegwu (2007)** and **Prakash *et al.*, (2008)** which showed a significant increase in liver enzymes in administered rats with CCl_4 alone. In addition, **Adewale *et al.*, (2014)** confirmed that the CCl_4 caused severe hepatic damage as evidenced by the significant increase in serum activity of AST, ALT and ALP enzymes and levels of MDA, as well as, the decrease in the activity of Glutathione-S-transferase (GST) and CAT enzymes in rats. Recently, numerous studies discovered a significant increase in serum levels of ALT, AST, ALP, Cr, UN, UA, Alb, MDA, TL, TC, TG, LDL-c and VLDL-c, even as serum GSH, GST, SOD and HDL were diminished in CCl_4 administered rabbits, compared to normal control **(Ahmad *et al.*, 2021)**. **Ellappan *et al.*, (2022)** and **Sun *et al.*, (2022)** showed that CCl_4 injected rats causes liver detriment as evidenced by the elevation in liver enzymes and bilirubin levels and decreased the SOD, CAT, and GSH levels. As confirmed by **EL Sayed *et al.*, (2019)** that the livers of received rats CCL_4 were influenced greatly, as characterized by the increase in the thickness and multiple vacuoles of different sizes and shapes in the capsule, as well as the thickening of connective tissue septa and loss of the normal hepatic architecture. Additionally, multiple, large cytoplasmic vacuoles with flattened peripheral eccentric nuclei between the hepatocytes.

The mechanism by which CCl4 causes hepatotoxic might be related to the cytochrome P-450-supporting metabolic initiation of to yield free radicals, which can bring lipid peroxidation. And therefore, this leads to the death of cells exposed to oxidative stress, the release of liver enzymes outside the hepatocytes, and markedly affected levels of MDA, SOD, and GSH (**Shehu *et al.*, 2022**). The rise of MDA level can reflect the injury of oxidative damaged cells. As well, the reduction in the antioxidant levels allows excessive production of

free radicals, followed by depletion of the extracellular antioxidant enzymes. Consequently, oxidative stress is a pivotal factor contributing to severe liver injury (**Sutti *et al.*, 2014**).

When comparing the injected rats with CCl_4 and fed on a complementary diet with the different proportions of GTNs (5, 10 and 15%), and those injected with CCl_4 and fed on the basal diet only. Our results discovered that rats treated with GTNs incorporated with CCl_4 injection have a notable increment in BWG, RBEG and FC. As well, the results revealed that GTNs has the ability to improve liver cells. The hepatoprotective performance of GTNs might be due to their ability either diminish the harmful effects or to preserve the normal hepatic physiological mechanism which has always been unbalanced by a hepatotoxic induced by CCl_4. Additionally, GTNs also provides the ability to curtail damage to kidney functions as evidenced by the improvement in serum Cr, UN and UA levels. It also had an effective role in reducing the serum level of TL, TG and TC. Moreover, it also turned out to have an effective role in diminishing the oxidative stress induced by CCl_4, via a significant decrease in the level of MDA and an increase in antioxidant enzymes in liver tissues.

Our results are in agreement with **Oyedepo and Odoje (2014)** who stated that tiger nuts substantially reduced serum levels of liver enzyme, and lipid peroxidation in liver tissues of treated rats with CCl_4. Also, these results were in concurrence with **Abiola and Mutiu (2020)** who indicated that tiger nuts exhibited hepatoprotective effects in experimental rats. **Seham *et al.*, (2017)** reported that hepatotoxic rats feeding on a formulated diet with tiger nut have a significant decline in serum levels of AST, ALT and ALP, TC, TG, UN, Cr, UA, total protein, Alb and globulin, as well as a rise in BWG and feed intake. Further, the obtained results are comparatively consistent with **Ibitoye *et al.*, (2018)** who indicated rats fed on the tiger nut oil- diet have a significant decrease in serum TC, TG, LDL-c, VLDL-c. In addition, **Innih *et al.*, (2021)** established that the treated rats with the tiger nut extract had a significant increase in SOD, CAT and GSH-Px activities and a reduction in MDA level when compared to the untreated cadmium-induced rats. **Adam *et al.*, (2019)** showed that the co-treatment of red Sokoto goat by lead acetate with methanol extract of tiger nut increased serum GPx, CAT

and SOD. Also, there was a lower MDA concentration in the similar group. Recently, **Mfem *et al.*, (2021)** revealed that tiger nut extracts possess antiatherogenic, antioxidant and hepatoprotective properties.

Thus, the hepatoprotective effect of tiger nuts may be due to its containment of several amounts of phytochemicals which exhibit several pharmaceutical and biological actions (**Amadi *et al.*, 2006**). The tiger nut has been displayed to have medicinal properties due to its content of bioavailable substances such as polyphenols, flavones, essential fatty acids, minerals and vitamins (C, D, E and B) (**Yu *et al.*, 2022**). It has been proven before that the tiger nut has good antioxidant characteristics and can be used as a source of natural antioxidants due to its content of flavonoids (**Jing *et al.*, 2015**). Moreover, **Amelia *et al.*, (2020)** mentioned that flavonoids, polyphenols, and vitamins like E and C possess notable antioxidant action and free radical clearance molecules. **Ademosun and Oboh (2015)** exhibited that the radical scavenging ability and the inhibition of lipid peroxidation and MDA production in the pancreas of rat in vitro. Additionally, the earlier findings supported that the tiger nut may be helpful in reducing oxidative stress and inflammation in the liver (**Achoribo and Ong, 2017**). **Iboyi *et al.*, (2021)** reported that tiger nuts are helpful in protecting the human body from several diseases due to their higher content of vitamin E, oleic acid and antioxidants. **Ezeh *et al.*, (2014)** also referred to tiger nuts as containing vitamins like C and E, and minerals such as potassium, calcium and magnesium as well as phenolic compounds.

Moreover, **Ogunlade *et al.*, (1997)** mentioned that tiger nuts contain protective nutrients because they have the proper amount of vitamins E and C, magnesium, copper, zinc and iron. Zinc has an important role in immunity. Copper aids in iron metabolism and works with a lot of antioxidant enzymes, particularly those implicated in protein metabolism. Vitamin E as informed by **Szymańska *et al.*, (2020)** is a strong antioxidant and accountable for inhibiting lipid peroxidation, which can lead to damage to cell membranes, proteins, and DNA in the body. Also, vitamin C is a strong reducing agent and acts as a scavenger of oxidizing free radicals and deleterious several varieties of oxygen-derived in the biological systems (**Pehlivan, 2014**). Additionally, Zinc acts as part of the critical antioxidant enzymes and protects cells from oxidative damage due to its ability in membrane

stabilization and restrains nicotinamide adenine dinucleotide phosphate oxidase enzyme. In addition, zinc participates in metallothionein synthesis, involved in the lowering of hydroxyl radicals and in the sequestration of the reactive oxygen species generated in the stress states (**Ruz, 2012**).

Tiger nut oil is an abundant source of essential fatty acids (EFA) and phytosterols (**Rita, 2009**). Oleic acid is the most prominent fatty acid in the oil and has an important role in regulating blood lipids and decreasing cholesterol levels (**Sobhani *et al*., 2018**) and TG, LDL-c, and increased levels of HDL-c and glutathione in rats (**Ibitoye *et al*., 2018**).

The germination process of tiger nuts increases the antioxidant activity (**David, 2005**) and the total polyphenol content (**Adebayo and Arinola, 2017**). Also, **Kaushik *et al*., (2010)** found that the germination improved the contents of vitamin C, niacin and riboflavin, as well as minerals (zinc, copper, calcium and manganese). **Iboyi *et al*., (2021)** confirmed that the germination process is associated with an increased concentration of vitamins and the bioavailability of trace minerals.

5. CONCLUSION

In conclusion, the current study was done to evaluate the potential effect of GTNs on the amelioration of some biochemical and histopathological markers in hepatotoxic rats induced by CCl_4. GTNS has shown notable enhancement in the hepatic and renal functions, serum lipid profile and antioxidant defense, as well as a pronounced development in liver tissue. Therefore, GTNs might mitigate the deleterious toxic effect of CCl_4. Nevertheless, further investigations are necessary to corroborate these effects of tiger nuts on human beings and to detect the specific components that have the beneficial properties.

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