Protective Effect of Tiger Nut (*Cyperus esculentus L.*) on Hypercholesterolemia Rats

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Abstract

The aim of the present work was to investigate the effect of tiger nut on rats suffering from hypercholesterolemia. Forty-eight male albino rats weighing 160 ± 5 g were used in this study. The rats divided into two main groups, the 1st main group (n=6) was fed on the basal diet (-ve control).The 2nd group (n = 6) was fed on hypercholesterolemic diet with addition of 1% cholesterol +0.5% colic acid+10% corn oil to induce hypercholesterolemia. Groups (3-5) were fed on basal diet supplemented with (10, 15, 25%) tiger nut, respectively. Groups (6-8) were fed on hypercholesterolemic diet supplemented with (10, 15, 25%) from tiger nut. The results revealed that all hypercholesterolemic groups which treated with 10%, 15%, and 25% of tiger nut resulted a varied increase in body weight gain, feed intake and feed efficiency ratio. Results declared that there was a significant difference (P≤0.05) among the positive control group and cholesterol groups treated with the different levels of tiger nut in internal organ weights. On the other hand, data showed that there was no significant difference (P≥0.05) in organs weight between the negative control group, and the group which treated with 25% tiger nut rats group. The results showed a significant decrease in AST, ALT, ALP enzymes activity, creatinine, blood urea and uric acid for treated groups as compared with positive control group. Results indicated that hypercholesterolemic rat groups, which treated with 10,15 or 25% tiger nut resulted in significant decreases (P≤0.05) in the values of serum total cholesterol, T.G, LDL-cholesterol, VLDL and LDL-cholesterol but showed mostly significant increase (P≤0.05) in the values of serum HDL-cholesterol compared to control (+ve) group. Tiger nut also, lowered malondialdehyde, while raised glutathione in serum. So, the study recommended using tiger nut in diets to overcome the problems of hypercholesterolemia and also improving the liver and kidney functions.

Key words: Cholesterol- Tiger nut – Liver function-Kidney function.

Introduction

Hypercholesterolemia is amongst the most common conditions encountered in the medical profession. It remains one of the key modifiable cardiovascular risk factors, and there have been recent advances in the methods and treatment options available **(Soran et al., 2018).** Hypercholesterolemia is a metabolic disorder caused by an increase in the concentrations level of plasma low-density lipoprotein (LDL) cholesterol. It has been implicated as a primary risk factor related to the pathogenesis of atherosclerosis or coronary heart disease, ischemic heart disease or cardiovascular disease, including myocardial infarction **(Tayo et al.,2018).**

Cholesterol is a waxy, fat-like substance found in the blood and body cells of all humans and animals. It falls under one of the three major groups of lipids which are manufactured and utilized to build membranes in all kinds of animal cells. It also serves as a precursor for the production of steroid hormones, vitamin D and bile acids, it is the main of sterol in the tissues of all animals **(Vazhacharickal et al., 2017).**

In recent years' new food products with increased health benefits and potential to reduce risk of diseases have been developed and marketed, as a result of advances in food technology and nutritional sciences. The dietary concerns of both today’s ageing population and people with fast food lifestyles have moved from foods that prevent nutritional deficiency and associated diseases to foods that offer longer-term prevention of chronic diseases. Countries are currently faced with health challenges arising from changing population demographics (e.g. an ageing population) and increases in lifestyle-related diseases. Consumers are becoming more aware of the relationships between diet and disease **(Sun-Waterhouse, 2011).**

Tiger nut is cultivated in Europe, South America, Asia, and Africa. Many varieties are cultivated, but only yellow and brown varieties are readily available for public consumption. Many preferred yellow varieties to others because of its attractive color, bigger size, and fleshier nuts. It also produces more milk upon extraction, contains more proteins, and possesses less anti-nutritional factors like polyphenols and lower fat (**Musa and Hamza, 2014).** Tiger nut is considered to possess some therapeutic effects **(Farre, 2003& Bixquert, 2003).**

Tiger nut is rich in starch, oil, minerals, and vitamins E and C. The starch and oil are major macronutrients in the tiger nut tuber. High starch content of this plant provide unique functional properties **(Manek et al., 2012),** cold storage stabilities, and preserves organoleptic properties of foods **(Jing et al.,**

2012). The tiger nut oil also has high monounsaturated fatty acids, similar to olive, avocado and hazelnut oil**.** These monounstaturated oil has high unsaponifiable matter, phospholipids and other bioactive compounds such as tocopherols, phytosterols and polyphenols **(Ezeh et al., 2014).**

Although tiger nut oil fatty acid profile is similar olive oil, nut oil has unique gold-yellow color, neutral taste properties, high in phytosterols **(Sanchez-Zapata et al., 2012)**, and better deep frying stability **Lasekan and Abdulkarim, 2012)** .The nutritional profiles and unique functional properties have made tiger nut products as unique foods **(Ekeanyanwu and Ononogbu, 2010)** like beverage, flour (**Oladele and Aina, 2007; Chinma et al., 2010),** edible oil **(Muhammad et al., 2011; Lasekan and Abdulkarim, 2012),** and as a feed source **(Sanchez-Zapata et al., 2012).**

Therefore, this study aimed to investigate the effect to tiger nut on nutritional parameters, liver enzymes, kidney functions, lipid profile, malondialdehyde and glutathione of rats suffering from htpercholesterolemia.

Materials and Methods

Materials:

Tiger nut (*Cyperus esculentus* Lam), was obtained from Agriculture Research Center, Egypt, then milled to get the powder (National Research Center, Giza, Egypt).

Rats:

Adults male albino rats (n = 48) of Sprague- Dawely strain weighing (160 ± 5 g) were purchased from Helwan Farm of Experimental Animals, Helwan, Egypt.

Chemicals:

Casein, vitamins, minerals and cellulose were obtained from Elgomhoria Company, Egypt. Kits were purchased from Gama Trade Company, Dokki, Egypt. Cholesterol was obtained from VACSERA, Dokki, Egypt

Methods:

Determination of Nutritive value:

The proximate composition of tiger nut powder was determined by the various methods described by **AOAC, (2010)**.

Biological Experiment:

A group of Forty-eight rats were housed in hygienic conditions and fed on basal diet **(Reeves *et al.*, 1993)** for one week for adaptation. After this week, rats were randomly divided into 8 groups. The 1st group (n=6) was fed on the basal diet (-ve control). The 2nd group (n = 6) was fed on Hypercholesterolemic diet (basal diet with addition of 1% cholesterol +0.5% colic acid +10% corn oil to induce hypercholesterolemia). Groups 3, 4 and 5 were fed on basal diet containing 10%, 15% and 25% tiger nut, respectively. Groups 6-8 were fed on hypercholesterolemic diets supplemented with (10%, 15% and 25% tiger nut), respectively.

Blood Samples Collection:

At the end of the feeding trail (12 weeks), animals were fasted over –night, lightly anesthetized under ether. Blood was withdrawn into clean dry centrifuge plastic tubes. Blood samples were centrifuged and sera obtained then stored at -20ºC in a clean well stopped vial until analysis.

Feeding and growth parameters:

Feed intake (FI), feed efficiency ratio (FER), body weight gain percent (BWG %) and organs relative weight were calculated according to **Chapman *et al.*, (1959**

Biochemical analysis:

Alanine aminotransferase (ALT) and aspartate aminotranseferase (AST) were estimated according to **(Thomas, 1998).** Serum alkaline phosphatase (ALP) was determined according to **(Roy, 1970).** Serum creatinine (Cr) was determined according to the method described by **Henry (1974)**. Serum urea concentration was determined according to the method described by **Fossati et al., (1980)**. Serum malondialdehyde (MDA) and glutathione were measured according to (**Sinha, 1972, and Draper and Hadly, 1990**) respectively. Total cholesterol (TC), Triglyceride (TG) and High density lipoprotein (HDL) was carried out according to **(Meiattini, 1978; Fossati and Praneipe, 1982 and Young, 2001)** respectively. VLDL-c and LDL-c were calculated as described by **Friadwald et al., (1972).**

Statistical analysis:

The data were analyzed according to SPSS program. ANOVA test was used to compare among groups and $P<0.05$ was considered to be significant **(SPSS, 1986).**

Results and Discussion

Chemical Composition of Tiger Nut:

Table (1): Chemical composition of Tiger Nut (g/100g)

Nutrients		%
Nutrients (g)	Proteins	8.70
	Fats	34.43
	Carbohydrate	42.22
	Fiber	6.62
	Moisture	3.78
	Ash	4.25
Vitamins (mg)	Vitamin E	42.9
	Vitamin C	30.7
Minerals (mg)	Zinc	0.01
	Iron	0.80
	Potassium	255
	Magnesium	56.30
	Calcium	140
	Phosphorus	121
	Copper	0.01
	Sodium	235

The data of chemical analysis of tiger nut are presented in table (1). The moisture, protein, carbohydrate, ash and fiber contents of tiger nut were 3.78%, 8.70%, 42.22%, 4.25 % and 6.62%, respectively. Concerning the oil content of tiger nut, it was clear that tiger nut contained high amount of fat, being 34.43%. Hence, data shows that the tiger nut could be considered as a source of oil. Thereafter, it could be concluded that tiger nut is a good source of oil and carbohydrate which may be useful for human nutrition. The obtained findings were nearly similar with those obtained by **Muhammad et al., (2011); Adejuyitan (2011); Imam et al., (2013) and Idoia Codina et al., (2014).** While micronutrient (mineral and vitamin) were as following, magnesium (Mg)56.3 mg, potassium (K) 255 mg, phosphorus (P) 121 mg, calcium (Ca) 140 mg, sodium (Na) 235 mg, copper (Cu) 0.01 mg, iron (Fe) 0.80 mg and zinc (Zn) 0.01 mg. The vitamin E value obtained is 86.73 (µg) and vitamin C concentration is 30.70 mg.

Belewu and Abodunrin (2006) found that tiger nut is high in starch, glucose, and proteins, contain high amounts of minerals elements like phosphorous and potassium, as well as in vitamins C and E. It equally contains a high amount of oleic acid which can defend the internal mechanisms and prevent constipation, diarrhea and cardiac disorders. Consumption of tiger nut milk has not been associated with any form of allergy. **Aremu et al., 2016** found that tiger nut was rich in meristic acid, oleic acid and linoleic acid.

Tiger nut contains high amounts of nutrients, vitamins, fiber, antioxidants, monounsaturated fatty acids and amino acids that make it suitable to be part of anti-cancer diets. It also contains elements proven to have anticancer properties (Quercetin, ß-sitosterol, fatty hydroxamic acid, oleic acid, vitamin D and E). In addition to its role in preventing hepatic oxidative stress, it exhibits anti-inflammatory effects against atherosclerotic lesions, mediated by accumulation of inflammatory cells into the inflammatory lesions in blood of mice **(Elom and Ming, 2017).**

Table (2): Protective Effect of Tiger Nut on Body Weight %, Feed Intake and Feed Efficiency Ratio of Rats with Hypercholesterolemia

Parameters / Groups	Body weight Gain (%)	Feed intake (g/day/rat)	Feed efficiency ratio
Control (-Ve)	126.00 ±5.43[a]	9.97 ±0.39[a,b]	12.62 ±0.42[c]
Control (+Ve)	104.80 ±17.95[f]	6.20 ±0.53[c]	9.68 ±2.02[d,e]
Tiger nut (10%)	120.40 ±4.82[b]	10.88 ±0.55[a]	19.54 ±2.38[a]
Tiger nut (15%)	120.20 ±11.52[b]	9.91 ±0.46[a,b]	12.12 ±0.85[c]
Tiger nut (25%)	125.20 ±11.12[a,b]	8.83 ±0.57[b]	14.24 ±1.80[b]
Hypercholesterolemia diet+ Tiger nut (10%)	116.20 ±8.58[c]	10.74 ±0.40[a]	10.67 ±0.71[d]
Hypercholesterolemia diet+ Tiger nut (15%)	112.80 ±15.43[d]	8.92 ±0.69[b]	12.80 ±2.69[c]
Hypercholesterolemia diet+ Tiger nut (25%)	109.00 ±16.73[e]	8.06 ±0.38[b,c]	13.59 ±2.52[b,c]

*Values are expressed as means ±SE.

*Values at the same column with different letters are significantly different at P<0.05

As shown in table (2), data indicated that body weight gain % for the (+ve) control group was significantly decreased (P<0.05), compared to the negative control group (104.80±17.95 VS 126±5.43%), respectively. The reduction noticed in body weight gain, feed efficiency, feed intake and growth rate for rats fed hypercholesterolemic diet may be due to the decreased palatability of dietary mixture **(El-Sayed et al., 2004),** where pure cholesterol and animal fat were added increased the energy density of the diet leading to decreased feed consumption **(Nwodo et al., 2014)**. BWG% increased significantly (P<0.05) in hypercholesterolemic groups which fed on different levels of tiger nut compared to the positive control one. The highest BWG % was observed in group of rats fed on basal diet supplemented with tiger nut at level 25% with a mean value of 125.20 ± 11.12%. Feed intake was decreased in the positive control group compared to protective and hypercholesterolemic groups. The results indicated that feed intake was increased in groups Fed on diets containing different levels of tiger nut, as compared to the positive control group. FER was significantly ($P<0.05$) decreased in the +ve control group compared to the (–Ve) control with a mean value of 9.68 ±2.02 VS 12.62 ±0.42, respectively. There were significant changes in FER among healthy and Hypercholesterolemic groups fed on all different levels of tiger nut compared to the negative control group.

Consequently, supplementing diets with tiger nut enhanced body weight gain, feed intake and feed efficiency ratio at all levels of supplementation compared with the positive control group. Furthermore, it has been recognized that taste and palatability of the diet affected feed intake. The results indicate that increasing tiger nut proportion in hypercholesterolemic rat diet was accompanied with the body weight gain, feed intake and growth rate increasing.

The increasing of body weight gain, feed intake and feed efficiency in treated rat groups may be due to the higher content of phytochemical compounds and monounsaturated fatty acids found in tiger nut more than of corn oil, and its effect on protein metabolism by activation some enzymatic pathway, the activity effect caused increasing in protein absorption and consequently an increase in weight gain, feed intake and feed efficiency ratio **(Li et al., 2011).** The obtained results are in agreement with those reported by **Ghasi et al., (2000); Bamishaiye et al., (2010); Anthony and Ashawe, (2014); Oseni et al., (2015); Atef and Al-Rethea (2016) and Mohamed (2016).**

Table (3): Effect of Tiger Nut on Relative Organs Weight of Rats with Hypercholesterolemia

Groups / Parameters	Liver weight body weight %	Kidney weight body weight %
Control (-Ve)	4.96 ±0.29 b	5.46 ±0.16 a,b
Control (+Ve)	6.66 ±0.27 a	6.30 ±0.20 a
Tiger nut (10%)	5.30±0.46 a,b	5.90 ±0.45 a,b
Tiger nut (15%)	5.16±0.21 a	5.56 ±0.11 a,b
Tiger nut (25%)	6.16 ±0.27 a	6.00 ±0.30 a
Hypercholesterolemia diet+ Tiger nut (10%)	5.88 ±0.44 a,b	5.92 ±0.33 a,b
Hypercholesterolemia diet+ Tiger nut (15%)	6.50±0.57 a	5.92 ±0.27 a,b
Hypercholesterolemia diet+ Tiger nut (25%)	5.42 ±0.44 a,b	5.68 ±0.41 a,b

.*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at $P<0.05$.

The result of changes of the relative organs weight is shown in table (3). Rats group of the positive control group fed on hypercholesterolemic diet had increases in the mean values of relative liver and kidney weight as compared to the (–ve) group. Hypercholesterolemia diet with supplemented tiger nut, caused reduction in the mean value of relative liver and kidney weight compared to the +ve control except rats group fed on basal diet containing tiger nuts at levels 25% . This results are in agreement with **Mahmoud and Aly, (2015)** who reported that rats received 20% of tiger nut demonstrated an increase in relative weight of liver after 6 weeks of treatment.

Table (4): Protective Effect of Tiger Nut on Serum Liver Functions of Rats with Hypercholesterolemia

Parameters / Groups	ALT	AST	ALP
	(U/L)		
Control (-Ve)	27.40 ±2.8[f]	34.60 ±0.5[f]	806.80 ±4.08[g]
Control (+Ve)	76.40 ±1.8[a]	104.00 ±7.1[a]	868.00 ±1.00[a]
Tiger nut (10%)	35.00 ±1.4[d]	40.60 ±4.7[d]	834.20 ±3.83[d,e]
Tiger nut (15%)	35.20 ±3.0[d.]	42.40 ±5.5[c]	834.20 ±2.83[d,e]
Tiger nut (25%)	33.00 ±3.7[e]	38.80 ±3.0[a.b]	820.80 ±1.83[f]
Hypercholesterolemia diet+ Tiger nut (10%)	37.80 ±2.8[c]	45.60 ±4.1[b]	845.20 ±4.12[b]
Hypercholesterolemia diet+ Tiger nut (15%)	40.80 ±1.9[b]	45.60 ±2.3[b]	840.52 ±3.21[c]
Hypercholesterolemia diet+ Tiger nut (25%)	35.60 ±2.7[d]	39.00 ±2.9[d,e]	835.42 ±3.52[d]

.*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

Results in table (4) show the protective effect of tiger nuts on the activity of Alanine Aminotransferase (ALT). The group of rats were fed on cholesterol and colic acid (positive control group), had significantly increased level of ALT in serum with a mean value of 76.40 ±1.8 U/L compared with the negative control group (27.40±2.8 U/L). Results revealed that rats were fed on hypercholesterolemic diet supplemented with tiger nut showed reduction in the serum activity of ALT at any levels when compared with the positive control group. The lowest activity of ALT in serum was showed in protective rats were fed on tiger nut at level 25% with a mean value 33.00 ±3.7 U/L.

Protective effects of tiger nuts on serum activity of Aspartate Aminotransferase (AST) showed in table (4). Data indicated that AST activity revealed significantly increased when animals were fed on cholesterol and colic acid (positive control group) with a mean value of 104.00±7.1 U/L compared with the negative control group (34.60 ±0.5 U/L). However, when rats were fed on tiger nut in diet at any levels of intake showed significant decreased in the serum level activities of AST when compared with the positive control group.

The activity of serum ALP was significantly increased ($P<0.05$) in rats with hypercholesterolemic as compared with the corresponding values of normal group table (4). Feeding hypercholesterolemic group on tiger nuts resulted in a significant decrease ($P<0.05$) in serum ALP level, compared to the +ve group. Mostly there were no significant differences in the levels of serum ALT and AST due to supplementation level with tiger nuts.

The liver functional enzymes AST and ALT activities in experimental animal bloods are considered the excellent marker of liver dysfunctions and damages which caused by exposure to the toxic substances **(AbdEl-Ghany, 2006 and EL-Naggar, 2007)**. Results showed that after 12 weeks, the serum AST and ALT activities in treated rat groups were gradually decreased by increasing the concentration of tiger nut in the diet, compared with hypercholesterolemic rats group (+ve). Herein, data (Table 4) indicates that as tiger nut levels in hypercholesterolemia rat diet increased, the AST and ALT activities are decreased. Therefore, tiger nut may be enhancing the detoxicated properties of the liver. The enhancement in liver function enzymes activities with addition of tiger nut in the diet could be attributed to high content of monounsaturated fatty acid and phytochemicals. These benefits substances are providing the protection against the lipid oxidation in liver **(Badawy and Hegazi, 2004; Gambo and Da' u, 2014 and Mohamed, 2016).**

Tiger nut improved liver functional enzymes activity (Table 4). These results are in agreement with those of **Mohamed and El-Metwally (2004),** who proved that treatment with vitamin E significantly inhibited lipid peroxidation as well as liver cell damage. The decrease in serum AST and ALT activities for treated rat groups may be attributed to the decrease in oxidative stress through the antioxidants properties of Vit. E. Meanwhile, the increase in serum activities of AST and ALT for hypercholesterolemia rats group may be attributed to the toxic liver damage induced by cholesterolemic diet **(Mohamed, 2016).** The non-significant change in the activities of ALP, ALT, and AST in rats fed with tiger nut based diet compared to the control diet indicates unaltered tissue. This is so as these cellular enzymes are indicators of cellular integrity **(Ajiboye, 2015 and Ajiboye et al., 2017).** High fat diet could increase the body's metabolism burden via fatty depositions in the endothelial vascular system and can reduce the endothelial production of antioxidant enzymes, increased production of free radicals leading to an oxidative stress which damages the body's biological macromolecules, tissue and organ function disorders then ensues **(Aneckova, 2001)**.

Table (5): Protective Effect of Tiger Nut on Serum Kidney Functions of Rats with Hypercholesterolemia

Parameters / Groups	Urea	Creatinine	Uric acid
	mg/dl		
Control (-Ve)	35.40 ± 1.81[c]	0.80 ±0.02[c]	4.08 ±0.08 [g]
Control (+Ve)	42.80 ± 1.92[a]	1.30 ±0.10[a]	5.36 ±0.19 [a]
Tiger nut (10%)	38.40 ±0.89[b]	0.81 ±0.01[b,c]	4.30 ±0.27[e]
Tiger nut (15%)	37.20 ±1.92[b,c]	0.83 ±0.06[b]	4.26 ±0.13[e]
Tiger nut (25%)	35.80 ±1.92[c]	0.78 ±0.04[d]	4.70±0.20[b]
Hypercholesterolemia diet+ Tiger nut (10%)	33.80 ±0.83[d]	0.82 ±0.02[b]	4.38 ±0.25[d]
Hypercholesterolemia diet+ Tiger nut (15%)	37.60 ±2.07[b.c]	0.78 ±0.05[d]	4.22 ±0.14[e,f]
Hypercholesterolemia diet+ Tiger nut (25%)	37.00 ±2.34[b.c]	0.82 ±0.04[b]	4.54 ±0.20[c]

*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

Table (5) results show the protective effect of tiger nut on kidney functions (urea, creatinine and uric acid concentration in serum) of rats with hypercholesterolemia. When rats were fed on hypercholesterolemic diet the concentration of serum levels of urea were significantly increased with a mean value of 42.80 ± 1.92 mg/dl compared with the negative control group (35.40 ±.1.81mg /dl). While the group of rats fed on cholesterol ,colic acid and supplemented with tiger nut at any levels of intake, showed significant decrease in serum levels of urea to become as the normal levels compared with the positive control group. Also, the concentration of urea in serum were significantly decreased when rats were fed on basal diet and supplemented with tiger nuts the three levels of intake with mean values of 38.40 ±0.89 , 37.20 ±1.92 and 35.80 ±1.92 , respectively.

Data of table (5) show that the positive control group which fed on cholesterol, colic acid had increased in the concentration of creatinine with a mean value of 1.30 ±0.10 mg /dl compared with the negative control group (0.80 ±0.02 mg / dl). Group of rats were fed on hypercholesterolemia diet and supplemented with tiger nut showed significant reduction in the concentration of serum levels of creatinine compared with the positive control group.

Protective effect of tiger nut on uric acid concentration in serum of rats with hypercholesterolemia showed in table (5). Data indicated that uric acid revealed significantly increased in (positive control group) with a mean value of 5.36 ±0.19 mg/dl compared with the negative control group (4.08 ±0.08 mg/dl). However, when rats were fed on tiger nut in diet at any levels of intake showed significant decrease in the serum uric acid when compared with positive control group.

Creatinine, urea and uric acid are markers of kidney function **(Shivaraj et al.,2010)** .The result of this study in table (5) revealed that *C. esculentus* plays a beneficial role in mitigating renal dysfunction of rats to high fat diet and an indication of kidney dysfunction is observed as the levels of markers of kidney function (Creatinine, Urea and Uric acid) significantly elevated $p<0.05$ in positive control relative to the normal control and the treatment groups which fed on hypercholesterolemic diet. However, *C. esculentus* treated group significantly lowered the levels of markers of kidney function .*C. esculentus* has been shown to contain unsaturated fatty acid (**Siqun, et al.,2013 & Ezeh et al.,2014)** , and as reported by **Hassan,(2007)**. This effect may be attributed to the positive role of unsaturated fatty acids in preservation of glomerular filtration rate and effective renal plasma flow.

Stanley and Francis (2018) reported that C. esculentus oil plays a beneficial role in mitigating renal dysfunction induced by exposure of the rats to high fat diet (HFD) and low dose Streptozocin (STZ) and an indication of kidney dysfunction is observed as the levels of markers of kidney function (creatinine, urea, sodium and potassium) was significantly elevated $p<0.05$. However, *C. esculentus* oil (HFD+STZ+CEO) treated group significantly lowered the levels of markers of kidney function as well as reducing the relative kidney weight.

C. esculentus oil has been shown to contain unsaturated fatty acid (**Muhammad et al., 2011)** and as reported by **Hassan, (2007)** this effect may be attributed to the positive role of unsaturated fatty acids in preservation of glomerular filtration rate and effective renal plasma flow.

Table (6): Protective Effect of Tiger Nut on Serum Lipid Profile of Rats with Hypercholesterolemia

Parameters / Groups	TC	TG	HDL-c	LDL-c	VLDL-c
	(mg/dl)				
Control (-Ve)	123.60 ±6.8[d]	111.40±6.1[e]	41.00 ±2.2[a]	60.32 ±21.2[e]	22.28 ±1.2 [d]
Control (+Ve)	232.00 ±6.4[a]	194.60±4.8[a]	31.60 ±1.5[d]	161.48 ±7.2[a]	38.92 ±0.9 [a]
Tiger nut (10%)	131.00 ±3.9[b]	116.80±1.9[d]	41.60 ±2.5[a]	66.04 ±7.8 [d]	23.36 ±0.3 [c]
Tiger nut (15%)	130.00 ±3.7[c]	119.00±3.8[c]	35.00 ±1.2[c]	71.20 ±3.9[c]	23.80 ±0.7 [c]
Tiger nut (25%)	129.60 ±1.8[c]	117.80 ±1.9[d]	39.20 ±3.3 [b]	66.84 ±2.4[d]	23.56 ±0.3 [c]
Hypercholesterolemia diet+ Tiger nut (10%)	133.60 ±2.9[b]	133.60 ±2.9[b]	36.40 ±2.1[c]	70.48 ±5.2[c]	26.72 ±1.3 [b]
Hypercholesterolemia diet+ Tiger nut (15%)	133.40 ±11.0[b]	117.00 ±1.5[d]	38.40 ±1.8 [b]	71.60 ±7.9[c]	23.40 ±2.2[c]
Hypercholesterolemia diet+ Tiger nut (25%)	132.80 ±5.2[b]	115.40 ±3.8[d]	32.00 ±1.9 [d]	77.72 ±7.2[b]	23.08 ±0.7[c]

*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

Total cholesterol (TC), triglyceride (TG), very low lipoprotein cholesterol l(VLDL-C), low density lipoprotein cholesterol (LDL-C), Increased significantly in the positive control group, as compared to the negative control group, as seen in table (6). However, the level of serum high density lipoprotein cholesterol (HDL-C) was significantly (P<0.05) lowered. Diets supplemented with tiger nuts showed significant reduction (P<0.05) in the mean values of serum lipid profile. However, serum HDL-C was increased significantly (P<0.05), compared to the (+Ve) control group. Groups of rats with hypercholesterolemia and fed on tiger nuts revealed improving lipid profile compared with the positive control group (+Ve). These alterations suggest the importance of tiger nut based diet in the management or prevention of cardiovascular related disorder. This is consistent with previous studies demonstrating the importance of other seed and nut oil **(Al-Naqeep et al., 2011; Gossell-Williams et al., 2008 and Sahebkar et al., 2016).** The elevated level of HDLc could lower predisposition the atherosclerosis and cardiovascular diseases **(Chen et al., 2011).**

Table (7): Protective Effect of Tiger Nut on Serum Malondialdehyde and Glutathione of Rats with Hypercholesterolemia

Groups / Parameters	MDA (mg/dl)	GSH (mg/dl)
Control (-Ve)	65.20 ±6.05[d]	4.10 ±0.00[c]
Control (+Ve)	145.00 ±6.44[a]	2.56 ±0.54[e]
Tiger nut (10%)	71.20 ±1.92[c]	4.94 ±0.41[a]
Tiger nut (15%)	75.20 ±1.92[b]	3.94 ±0.47[c,d]
Tiger nut (25%)	73.60 ±0.54[b,c]	4.92 ±0.46[a]
Hypercholesterolemia diet+ Tiger nut (10%)	75.20 ±2.77[b]	3.54 ±0.55[d]
Hypercholesterolemia diet+ Tiger nut (15%)	73.60 ±3.20[b,c]	3.94 ±0.82[c,d]
Hypercholesterolemia diet+ Tiger nut (25%)	71.40 ±2.07[c]	4.54 ±0.51[b]

*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

Rats were fed on hypercholesterolemic diet exhibited significant reduction (P<0.05) in the mean value of glutathione activity (Table7) as compared to the normal control group. However, the mean value of malondialdehyde level was significantly (P<0.05) increased in the positive control group compared to the normal one.

Moreover, the mean values of glutathione activities were significantly (P<0.05) increased as a result of feeding rats on tiger nuts at levels 10,15 and 25%, compared to the corresponding values of the positive control group. However, the mean value of malondialdehyde activity significantly (P<0.05) decreased as a result of feeding hypercholesterolemic rats on tiger nuts, compared to the (+Ve) control group. The tested sample of tiger nuts had beneficial effects on glutathione activity as well as on the malondialdehyde levels.

Antioxidant enzymes and nonenzymatic antioxidants protect cells by detoxifying reactive oxygen species **(Ajiboye, 2010 and Ajiboye et al., 2014)**. We proposed that tiger nut might have raised the antioxidants, thus maintaining the cellular integrity. The reduced glutathione increased significantly in all hypercholesterolemia groups studied. This increase could protect the cellular integrity by scavenging reactive oxygen species (ROS).

A study on the antioxidant activity of tiger nut indicated that it could be utilized to "mop up" and scavenge free-radicals, generate essential metabolic body reactions and environmental pollutants **(Ogunlade et al., 2015).** It was also suggested that addition of tiger nut as a side dish and adjunct in traditional diets would probably alleviate the symptoms associated with neurodegenerative and cardiovascular diseases **(Ogunlade et al., 2015).** Besides, with its high content of vitamin E and Quercetin, tiger nut might help provide cellular protection against free radicals and exert cancer cell-specific inhibition of proliferation at the G1 phase (**Allouh et al., 2015 & Jeong et al., 2009).**

Lipid peroxidation is the outcome of altered redox balance, usually in favor of prooxidant generations **(Niki, 2009).** In this study it was thought that the elevated GSH observed could prevent peroxidation of polyunsaturated fatty acid component of membrane. The level of malondialdehyde, a product of lipid peroxidation, was not altered by tiger nut based diet when compared to the control diet. The implication is that the integrity (organization and function) of membrane are preserved possibly by the GSH.

References

AbdEl-Ghany, M. (2006): Studies on Egyptian Bean (Moringa) seeds as nonconventional source of edible oil. Ph.D. Thesis, Fac. of Agric., Al-Azhar Univ.

Adejuyitan, J. (2011): Tiger nut processing: Its food uses and health benefits. American J. of Food Technol., 6 (3): 197-201.

Ajiboye, T. (2010): Redox status of the liver and kidney of 2,2-dichlorovinyl dimethyl phosphate (DDVP) treated rats. Chemico-Biological Interactions, 185(3): 202–207.

Ajiboye, T.; Akinpelu, S.; Muritala, H.; Ogunbode, S.; Adeleye, A.; Oladiji, A. and Oloyede, O. (2014): Trichosanthes Cucumerina fruit extenuates dyslipidemia, protein oxidation, lipid peroxidation and DNA fragmentation in the liver of high-fat diet-fed rats. Journal of Food Biochemistry, 38(5):480–490.

Ajiboye, T. (2015): Standardized extract of Vitex doniana sweet stalls protein oxidation, lipid peroxidation and DNA fragmention in acetaminophen-induced hepatotoxicity. Journal of Ethnopharmacology,164: 273–282.

Ajiboye, T.; Ahmad, F.; Daisi, A.; Yahaya, A.; Ibitoye, O.; Muritala, H. and Sunmonu, T. (2017): Hepatoprotective potential of Phyllanthus muellarianus leaf extract: studies on hepatic, oxidative stress and inflammatory biomarkers. Pharmaceutical Biology, 55(1): 1662.

Allouh, M.; Daradka, H. and Ghaida, J. (2015): Influence of Cyperus esculentus tubers (Tiger Nut) on male rat copulatory behavior. BMC complementary and alternative medicine, 23: 15(1):331.

Al-Naqeep, G.; Al-Zubairi, A.; Ismail, M.; Amom, Z. and Esa, N. (2011): Antiatherogenic potential of Nigella sativa seeds and oil in diet-induced hypercholesterolemia in rabbits. Evidence-Based Complementary and Alternative Medicine.

Aneckova, R. (2001): The role of leptin in human physiology and pathophysiology. Physiological Research, 50:443-459.

Anthony, V. and Ashawe, D. (2014): The effect of *Moringa oleifera* leaf meal on the hematological parameters and the cholesterol level of rabbits. American Journal of Biological, Chemical and Pharmaceutical Science, 2 (3): 1 - 6.

Aremu, M.; Ibrahim, H. and Aremu, S. (2016): Lipid composition of black variety of raw and boiled tigernut (*Cyperus esculentus L*) grown in North-East Nigeria. Pakistan J. Nutr., 15: 427-438.

Association of Official Analytical Chemist (AOAC)(2010): Official methods of analysis (18th ed.). Washington DC.

Atef, M. and Al-Rethea, H. (2016): Chemo-protective effect of omega-3 fatty acids on thioacetamide. Induced hepatic fibrosis in male rats. Saudi Journal of Biological Sciences, 23: 217 – 229.

Badawy, I. and Hegazi, S. (2004): Effect of natural and synthetic sweeteners on serum lipids and glucose of alloxan diabetic rats. Egyptian Journal of Nutrition, 19 (1): 131 - 150.

Bamishaiye, E.; Muhammad, N.; and Bamishaiye, O. (2010): Assessment of biological value of tiger nut *(Cyperus esculentus*) tuber oil meal-based diet in rats. Annals of Biological Research, 1 (4): 274 - 280.

Belewu, M. and Abodunrin, O. (2006): Preparation of Kunu from unexploited rich food source: tiger nut (*Cyperus esculentus*). World J. Dairy Food Sci.; 1: 19-21.

Bixquert, M. (2003): Digestive aspects of tiger nuts. Pesca. Y. Alimentacion, 71: 85.

Chapman, D.; Gastilla, C. and Campbell, J. (1959): Evaluation of protein in food. I.A. Method for the determination of protein efficiency ratio.Can. J. Biochem. Pysiol., 37(32):679-686.

Chen, Q.; Reis, S.; Kammerer, C.; Craig, W.; McNamara, D.; Holubkov, R. and Kamboh, M. (2011): Association of anti-oxidized LDL and candidate genes with severity of coronary stenosis in the Women's ischemia syndrome evaluation study. Journal of Lipid Research, 52(4): 801–807.

Chinma, C.; Abu, J. and Abubakar, Y. (2010): Effect of tiger nut (Cyperus esculentus) flour addition on the quality of wheat-based cake. Int. J. Food Sci. Technol., 45(8):1746-1752.

Draper, H. and Hadley, M. (1990): Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol., 186: 421-431.

El-Naggar, E. (2007): Effect of different heat treatments on the physical, chemical and biological characteristics of some edible oils. Ph.D. Thesis, Fac. of Agric., Al-Azhar Univ.

Elom, S. and Ming, T. (2017): Tiger nut (Cyperus esculentus): sources of natural anticancer drug? brief review of existing literature. Euromediterranean Biomedical Journal, 12(19):091-094.

El-Sayed, S.; Hammam, A. an Attia, A. (2004): Effect of natural and synthetic coloring agents on some biological parameters in rats. Egyptian J. of Nutrition, 19 (3): 59 – 87.

Ekeanyanwu, R. and Ononogbu, C. (2010): Nutritive value of Nigerian tigernut (Cyperus esculentus L.). Agric. J., 5(5):297-302.

Ezeh, O.; Micheal, H. and Niranjan, K. (2014): Tiger nut oil (*Cyperus esculentus* L.): A review of its composition and physic-chemical properties. Eur. J. Lipid Sci. Technol., 166(7):783-794.

Farre, R. (2003): Nutritional and dietetic aspects of tiger nuts. Food Sci. Technol. Int., 11: 33-40.

Fossati, P.; Prencipe, L. and Berti, G. (1980): Enzymatic colorimetric method of determination of urea in serum. Clin. Chem., 6(18): 499-502.

Fossati, P. and praneipe, L. (1982): Triglycerides determination after enzymatic hydrolysis .Clin. Chem., 28:2077.

Friadwald, W.; Levy, K. and Fredrickson, D. (1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., 226:499-504.

Gambo, A. and Dau, A. (2014): Tiger nut (*Cyperus esculentus*): Composition, products, uses and health benefits – a review. Bayero Journal of Pure and Applied Sciences, 7 (1): 56 - 61.

Ghasi, S.; Nwobodo, E. and Ofili, J. (2000): Hypocholesterolemia effect of crude extract of leaf of *M. oleifera* in high fat diet fed wistar rats. J. of Ethanopharmacology, 69: 21 - 25.

Gossell-Williams, M.; Lyttle, K.; Clarke, T.; Gardner, M. and Simon, O. (2008): Supplementation with pumpkin seed oil improves plasma lipid profile and cardiovascular outcomes of female non-ovariectomized and ovariectomized Sprague-Dawley rats. Phytotherapy Research, 22 (7): 873–877.

Hassan, A. (2007): The Potential effects of Tiger nut Oil on Some Haemato-Biochemical Blood Indices in Male Albino Rats. The Egyptian Journal of Experimental Biology (Zoology), 3:49-54.

Henry, R. (1974): Creatinine measurement with colorimetric method. In Clinical Chem., 2(5): 525-534.

Idoia Codina, T.; Buenaventura and Antonio, J. (2014): Characterization and comparison of tiger nuts (*Cyperus esculentus* L) from different geographical origin, physicochemical characteristics and protein fractionation industrial crops and products. Available online at http:www.tiger nut. com.

Imam, T.; Aliyu, F. and Umar, H.; (2013): Preliminary phytochemical screening, elemental and proximate composition of two varieties of *Cyperus esculentus* (Tiger nut).Nigerian J. of Basic and Applied Sci., 2(4): 247-251.

Jeong, J.; An, J.; Kwon, Y.; Rhee, J. and Lee, Y. (2009): Effects of low dose quercetin: Cancer cell-specific inhibition of cell cycle progression. Journal of cellular biochemistry, 106(1):73-82.

Jing, S.; Yan, X.; Ouyang, W.; Xiang, H. and Ren, Z. (2012): Study on properties of Cyperus esculentus starch grown in Xinjiang, China. Starch-Starke, 64(8):581-589.

Lasekan, O. and Abdulkarim, S. (2012): Extraction of oil from tiger nut (Cyperus esculentus L.) with supercritical carbon dioxide (SC-CO2). LWT-Food Sci. Technol., 47(2):287-292.

Li, R.; Guo, W.; Fu, Z.; Ding, G.; Zou, Y. and Wang, Z. (2011): Hepatoprotective action of Radix *paeniae rubra*.aqueous extract against CCL4 – induced hepatic damage. Molecules, 16 (10): 8684 - 8694.

Mahmoud, E. and Aly, S. (2015): Effect of tiger nut tubers (Cyperus esculentus L.) supplementations on blood parameters of albino rats. Minia J. of Agric. Res. & Develop, 35(2): 233-247.

Manek, R.; Builders, P.; Kolling, W.; Emeje, M. and Kunle, O. (2012): Physicochemical and binder properties of starch obtained from Cyperus esculentus. Aaps Pharmscitech, 13(2):379-388.

Meiattini, F. (1978): The 4-hydroxybenzoate /4-aminophenazone Chromogenic System .Clin. Chem., 24(12):2161-2165.

Mohamed, E. and El-Metwally, E. (2004): Effect of some antioxidants on carbon tetrachloride induced liver injury. Egyptian Journal of Nutrition, 19 (3): 191 – 208.

Mohamed, M. (2016): Hypocholesterolemia effects of oil and moringa leave extract. M.Sc. Thesis, Fac. of Agric., Assiut Univ. Egypt.

Muhammad, N.; Bamishaiye, E.; Usman, L.; Salawu, M.; Nafiu, M. and Oloyede, O. (2011): Physicochemical properties and fatty acid composition of *C. esculentus* (tiger nut) tuber oil. Bioresearch Bulletin, 5: 51-54.

Musa, A. and Hamza, A. (2014): Comparative analysis of locally prepared 'KununAya' (Tiger nut milk) consumed by students of Kaduna State University, Kaduna, Nigeria. Sci. World J., 8(2):34-78.

Niki,E.(2009): Lipid peroxidation: physiological levels and dual biological effects. Free Radic. Biol. Med., 47(5):469-84.

Nwodo, N.; Nnadi, C.; Ibezim, A. and Mabah, C. (2014): Plants with hypolipidemic effects from Nigerian flora.Chapter 10, antioxidants, antidiabetic agents and human health, 241 - 255.

Ogunlade, I.; Adeyemi, A.; and Aluko, G. (2015): Chemical compositions, antioxidant capacity of Tiger nut (Cyperus esculentus) and potential health benefits. European Scientific Journal, ESJ., 11(10):19-23.

Oladele, A. and Aina, J. (2007): Chemical composition and functional properties of flour produced from two varieties of tiger nut (Cyperus esculentus). Afr. J. Biotechnol, 6(21): 2473-2476.

Oseni, O.; Ogunmoyole, T. and Idowu, K. (2015): Lipid profile and cardio-protective effects of aqueous extract of *M. oleifera* leaf on bromated induced cardio toxicity on Wister albino rats. European Journal of Advanced Research in Biological and Life Sciences, 3 (2): 52 - 66.

Reeves, R.; Nielsen F. and Fahey, G. (1993): AIN-93 purified diets for laboratory rodents .J. Nutr.,123(1):1939-1951.

Roy, E. (1970): Colorimetric determination of Co. St Louis. Toronto. Princeton, 1088-1273.

Sahebkar, A.; Beccuti, G.; Simental-Mendía, L.; Nobili, V. and Bo, S. (2016): Nigella sativa (black seed) effects on plasma lipid concentrations in humans: A systematic review and meta-analysis of randomized placebo-controlled trials. Pharmacological Research, 106: 37–50.

Sanchez-Zapata, E.; Fernández-Lopez, J. and Angel Perez-Alvarez, J. (2012): Tiger nut (Cyperus esculentus) commercialization: health aspects, composition, properties, and food applications. Compr. Rev. Food Sci. Food Saf., 11(4):366-377.

Shivaraj, G.; Prakash, B.; Shruthi, S.; Vinayak, V.; Avinash, A. and Sonal, N. (2010): Markers of renal function tests. N. Am. J. Med. Sci., 2(4):170-173.

Sinha, A. (1972): Colorimetric assay of catalase enzyme. Anal. Biochem., 47: 389-394.

Siqun, J.; Weiqi, O.; Zhiyan, R.; Hengxu, X. and Zexin M. (2013): The Invitro and In vivo Antioxidant Properties of Cyperus Esculentus Oil from Xinjiang, China. J. Sci. Food Agric., 93:1505-1509.

Soran, H.; Adam, S.; Mohammad, J. and Paul, N. (2018): Hypercholesterolaemia - practical information for non-specialists. Arch. Med. Sci., 14(1):1-21.

SPSS, (1986): Statistical package for social science, version 18. SPSS Inc., II.USA.

Stanley, N. and Francis, O. (2018): The effect of *Cyperus esculentus* (Tigernut) oil on liver, kidney and hematological biomarkers in low dose streptozocin and high fat diet exposed male wistar rats**.** International Journal of Food Science and Nutrition**,** 3(4): 148-152.

Sun-Waterhouse, D. (2011): The development of fruit-based functional foods targeting the health and wellness market: A review. International Journal of Food Science and Technology, 46: 899-920.

Tayo, A.; Sidiqat, A. and Raphael T. (2018): Anti-hypercholesterolemic effect of unripe Musa paradisiaca products on hypercholesterolemia-induced rats. Journal of Applied Pharmaceutical Science, 8(10): 090-097.

Thomas, L. (1998): Alanine aminotransferase (ALT), aspartate amino transeferase (AST).Clinical Laboratory Diagnostic .1sted.Frankfurt: TH-Books Verlagsgesellschaft, 55-65.

Vazhacharickal, P.; Mathew, J.; Mohanan, R. and Mol, M. (2017): Phytochemical profiling of Garcinia gummi-gutta (Malabar tamarind) and in vitro analysis of cholesterol lowering effect.

Young, S. (2001): Effect of diseases on Clinical Lab .Tests 4th ad AACC.

ملخص البحث

التأثير الوقائى لحب العزيز على الفئران المصابه بارتفاع مستوى الكولسترول

أجريت هذه الدراسة لمعرفة التاثير الوقائى لحب العزيز على الفئران التى تعانى من ارتفاع مستوى الكوليسترول. تم استخدام ٤٨ من ذكور الفئران من سلالة الالبينو يتراوح وزنهم (١٦٠±٥ جرام).تم تقسيم الفئران الى مجموعتين اساسيتين . المجموعة الاولي وهي المجموعة الضابطة السالبة وتتغذي علي الغذاء الاساسي فقط . المجموعة الثانية تغذت على الغذاء الاساسى بالاضافه الى ١%كوليسترول+٠٥ حمض الكوليك لاحداث ارتفاع فى مستوى الكوليسترول للفئران. المجاميع من (٣-٥) تغذت علي الغذاء الاساسي فقط بالاضافه الى (٢٥,١٥,١٠%) من حب العزيز على التوالى. المجاميع من(٦-٨) تم تغذيتهم على الغذاء العالى فى مستوى الكوليسترول مع اضافة (٢٥,١٥,١٠%) من حب العزيز على التوالى لمدة ١٢ اسبوع. تشير نتائج تحليل الدم الى ارتفاع كلا من معدل الزيادة فى الوزن ،معدل تناول الطعام ومعدل كفاءة الطعام فى كل المجاميع التى تغذت على الغذاء العالى فى مستوى الكوليسترول عن المجموعه الضابطه السالبه. اوضحت النتائج ان هناك اختلاف فى الوزن الداخلى للاعضاء فى المجموعه الضابطه الموجبه والمجاميع التى تغذت على الكوليسترول و(١٠,١٥,٢٥%) من حب العزيز.بينما لم يكن هناك اختلاف معنوى بين المجموعه الضابطه السالبه والمجاميع الصحيه.اظهرت النتائج انخفاض معنوى فى انزيمات AST,ALT, اليوريا والكرياتينين وحمض اليورك فى المجاميع التى تمت معالجتها مقارنة بالمجموعة الضابطه الموجبه .اظهرت النتائج انخفاض فى قيم كلا من (الكولسترول الكلى ،الدهون الثلاثيه ،البروتينات ذات الكثافة المنخفضة والبروتينات ذات الكثافة المنخفضة جدا بينما كان هناك غالبا ارتفاع ملحوظ فى قيم البروتينات المرتفعه الكثافة للفئران المصابه بارتفاع مستوى الكولسترول وتغذت على الثلاث مستويات المستخدمه من حب العزيز فى الدراسة . وقد ادت التغذيه على حب العزيز ايضا الى انخفاض مالون داى الدهيد وارتفاع الجلوتاثيون فى السيرم.التركيب الكيميائى للأحماض الدهنية لحب العزيز مناسب للدفاع والحد من تدهور حاله الدهون .لذلك اوصت الدراسة باهميه استخدام حب العزيز فى الغذاء للتغلب على مشاكل ارتفاع مستوى الكوليسترول وايضا تحسين وظائف الكلى والكبد.

الكلمات المفتاحية: الكولسترول – حب العزيز – وظائف الكلى –وظائف الكبد.