

Hepatoprotective effect of fennel and tiger nut on biochemical parameters and DNA damage in rats

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Abstract

The present study was carried out to investigate studies of dried fennel and tiger nut in normal and on carbon tetrachloride (CCl₄) induced DNA damage using one concentration of each plants alone for 30 days in male albino rats.

A total of thirty male albino rats (150-200 g) were used in this study. Rats were divided into 6 groups each of 5 animals. Group one was kept as a control –ve and fed on basal diet only, while the two other groups were fed on basal diet mixed with grind dried fennel and tiger nut at concentration (5 g / 100 g diet) for 30 successive days. Other three groups were subcutaneous injected of CCl₄ (0.1 ml/100 g b.wt. for two weeks) to induced DNA damage change .One of these group was left as a control +ve (subcutaneous injection of CCl₄), where the other two groups were fed on basal diet mixed with grind dried fennel and tiger nut at concentration (5 g / 100 g diet) for 30 successive days.

At the end of experimental period, blood samples were collected from each rat for biochemical analysis and rats were then sacrificed to elucidate DNA damage in hepatocytes. Subcutaneous injection of CCl₄ caused significant increase in serum levels of AST, ALT, ALP, creatinine, urea, triglyceride, glucose, total cholesterol, LDL and lipid peroxide (MDA) while the levels of serum total protein, albumin, globulin, A : G ratio and glutathione transferase (GST) were significantly decreased .The smear on agarose gel had been observed in CCl₄ treated groups indicating random DNA fragmentation and a hallmark of necrosis . Fennel and tiger nut significantly restored the serum levels of biochemical parameters directed toward normal as compared with the control +ve group (injected with CCl₄).

Introduction

Plant foods not only represent the major source of nutrients for human, but also contain protective factors against chronic disease, coronary heart disease, antinstagenic and cancer. Plants are include some oral of the following characteristics, excellent source of Omega-3 fatty acids; rich sources of antioxidant and vitamin (alpha tocopherol, ascorbic acid); rich in glutathione, protein (**Sripanidkulchai et al. 2002 and Simeonova et al. 2001**).

Foeniculum vulgare commonly known as fennel is a well-known and important medicinal and aromatic plant widely used as carminative, digestive, lactagogue and diuretic

and in treating respiratory and gastrointestinal disorders. Its seeds are used as flavorings in baked goods, meat and fish dishes, ice cream, alcoholic beverages and herb mixtures. *F. vulgare* has been reported to contain 6.3% of moisture, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fiber and 42.3% carbohydrates. The minerals and vitamins present in *F. vulgare* are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C (Manzooret al.2012).

Tiger nut (*Cyperus esculentus* L.) is an edible perennial grass-like plant native to the Old World, and is a lesser-known vegetable that produces sweet nut-like tubers known as “earth almonds” (Coskuner et al . 2002). The nuts are valued for their highly nutritious starch content, dietary fiber, digestible carbohydrate (mono, di and polysaccharides) (De Vries 1991), sucrose, fat and protein, which are resistant to peroxidation (Oderinde and Tairu 1988). The nut is also fairly rich in mineral content (Sodium, Calcium, Potassium Magnesium, Zinc and traces of Copper) (Rita 2009).

Carbon tetrachloride (CCl_4) intoxication in animals is an experimental model that mimics oxidative stress in many pathophysiological situations (Mc Gregor and Lang, 1996). Carbon tetrachloride toxicity has resulted in many cases of poisoning by inhalation, ingestion or absorption. Prolonged exposure to carbon tetrachloride induced histopathological features such as inflammatory leucocytic infiltration, necrosis, fibrosis, cirrhosis and sometimes may lead to tumors (Qiu et al. 2005).

Jaramillo-Juárez et al. (2008) found that poisoning by CCl_4 induced toxic injury to both liver and kidney. Hepatic damage may be overshadowed by acute renal tubular necrosis, leading to renal oliguria of many species. Various studies demonstrated that CCl_4 intoxication caused free radical generation in many tissues such as liver, kidney, heart, lung, brain and blood. Ogeturk et al. (2005) reported that exposure to CCl_4 causes acute and chronic renal injuries.

The aim of the present study was to examine the protective effect of dried Fennel (*foeniculum vulgare*), and Tiger nut (*cyperus esculentus*) on biochemical analysis of serum blood samples in normal and carbon tetrachloride (CCl_4) exposure animal. Determination the DNA fragmentation in liver changes in normal and on carbon tetrachloride (CCl_4).

Materials and Methods

Plants material:

Fennel (*Foeniculum vulgare*) and Tiger nut (*Cyperus esculentus* L.) are obtained Local – market as dried powder. A voucher sample was kept in dry clean plastic bags, in the department of biochem, Toxicol&feed deff. AHRI.

Carbon tetrachloride (CCl_4):

Carbon tetrachloride (99.9 purity) was purchased from Sigma Chemical Company. It was used as 50% in 1 propylene glycol to rats at dose (0.1 ml /100 g b.wt) twice / week subcutaneously according to **Borah et al. (2004)**.

Animals:

Thirty white Albino rats of an average body weight 150- 200g were used for the experiment. They were obtained from the laboratory of animal colony, Ministry of Health and population, Helwan, Cairo, Egypt. Animals were acclimatized to laboratory condition before being used. Rats were fed on standard diet supplying the essential vitamins, trace elements and water, supply was given adlibitum.

Methods:

Experimental design:

This experiment was planned to study the protective effect of fennel (*Foeniculum vulgare*) and tiger nut (*Cyperuses culentus L.*) on DAN damage and some serum parameters in rats. For this purpose 30 mature rats were divided into 6 equal groups of 5 rats each for 30 successive days.

The groups were divided as follow:

Group 1: Kept as control negative and fed on basal diet

Group 2: Kept as control positive, feed on basal diet and was injected subcutaneously of CCl₄ by (0.1ml /100gb.wt.) S/C twice /2week for 30 successive days.

Group 3: Fed on basal diet mixed with dried fennel 5 g/100g of ration.

Group 4: Fed on basal diet mixed with dried tiger nut 5g/100g of ration.

Group 5: Fed on basal diet mixed with dried fennel 5 g/100g of ration after S/C injected of CCl₄ at a dose (0.1ml /100gb.wt.) S/C twice /2week for 30 successive days.

Group 6: Fed on basal diet mixed with dried tiger nut 5 g/100g of ration after S/C injected of CCl₄ at a dose (0.1ml /100gb.wt.) S/C twice /2week for 30 successive days.

Table (1) shows the result of ration analysis which used in feeding of rats. Ration was free of aflatoxin.

Table 1: Composition of ration (g/100g)

Constituents	Ratio %
Crude protein	14.6
Crude fiber	3.6
Moisture	2.18
Ash	10.7
Aflatoxin	None
Acid number of fat	7.2

Chemical composition of plant:

Moisture content, crude protein and ash content were determined according to the described by **AOAC (1995)**, the fat content according to **Horwitz (1980)**, crude protein **Less (1975)**, and vitamin E according to published procedure **Egyptian pharmacopeia (1984)**.

Samples:

1- Serum samples: Blood samples was obtained from the orbital plexuses of each animals and received into dry clean tube. Samples were left to clot at room temperature for about 2 hours, stored overnight in a refrigerator at 4 °C and centrifuged at 3000r.p.m.for 15 min . Serum samples were drawn in dry clean capped bottles and kept in a deep freeze for serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (**Reitman and Frankel 1957**),alkalinephosphatase(ALP) (**Roy1970**);total protein (**Sonnenwirth and Jaret 1980**); albumin (**Young, 1995**); creatinine (**Faulkner and King 1976**);triglycerides (**Knight et al. 1972**); total cholesterol (**Fredrikson et al. 1967**);serumHDL-Cholesterol(**N.C.E.P.1995**); serum glucose (**trinder, 1969**).Serum LDL- Cholesterol concentration was calculated according to **Friedewadet al. (1972)** formula. Serum Globulin was calculated by subtracting serum albumin from serum protein level. A/G ratio was calculated by divided serum albumin on serum globulin level. Gluathione-s-transferase (GST) (**Habig and Pabst 1974**);Lipid peroxide (Malondialdehyde) (**Ohkawa et al. 1979**). The animals were fasted for 12hr prior to the sacrificing.

2- Determination of DNA:

The extent of DNA fragmentation (DNA Ladder) has been assayed by electrophoresing genomic DNA samples, isolated from normal as well as experimental rat liver, on agarose /ethidium bromide gel by the procedure described by **Sellins and Cohen(1987)**.

Statistical analysis:

Parametric data were statistically analyzed by using Analysis of Variance (ANOVA) test and comparative of means were performed according to least significant differences test (LSD) according to (**Snedecor, 1969**)using **SPSS (2006)** . Results were presented as Mean \pm S.E.

Results and Discussion

The present study was carried out to elucidate the effect of fennel and tiger nut in normal and on carbon tetrachloride (CCl₄) induced DNA damage and some serum parameters using one concentration of each plant alone for 30 days in rats. The tested parameters were chemicals constituents, some biochemical parameters, and determination of DNA damage in liver in normal and injected rats by CCl₄.

It is generally believed that carbon tetrachloride (CCl_4) has hepatotoxicity, results from the bioactivation of the CCl_4 molecules to the trichloromethyl which is toxic free radical induced by certain isozymes of cytochrome P450 ($\text{CYP} - 450$). Once the trichloromethyl radical is formed, it reacts with molecular oxygen to form the highly toxic peroxy radical which then attacks cell membrane lipids to propagate a chain reaction leading to initiation of lipid peroxidation and break down of membrane structure (**Wong et al., 1998 and Youssef, 2000**).

The chemical constituents of fennel and tiger nut recorded in table (2). The record results are in agreement with **Manzoor et al. (2012)** and **Hwang (2004)**.

Body weight gain and food conversion:

Effect of feeding on ration mixed with each of fennel and tiger nut (5 g /100 g ration) for 30 successive days with or without subcutaneous injection of carbon tetra-chloride (twice/week for two weeks) on food intake and body weight gain are recorded in table (3). Body weight gain was significantly increased in rats given (5 g/100 g .diet) when compared with other groups. The group given tested plants with CCl_4 improved the body weight gain and food intake. These findings agreement with **Bamishaiye et al.(2010)**. **Ibrahim et al. (2013)**,who recorded improvement body weight gain, feed conversion values. Subcutaneous injection of CCl_4 caused a significant decrease in body weight gain and food intake. These results are in agreement with **Lee et al. (2007)**. **Aneja et al., (2005)** who concluded that feeding rats with antioxidants or flavonoids could play an important role as a prophylactic against the toxic effects of CCl_4 .

Hepatoprotective activity in carbon tetrachloride model:

Table (4) represented that rats fed on ration mixed with tiger nut for 30 successive day showed insignificant changes in the concentration of lipid peroxidation but fennel increasing level to compare with control -ve group. On the other hand, feeding rats on ration mixed with fennel and tiger nut showed highly significant increasing in the concentration of glutathione transferase. Feeding of tested plants with injected CCl_4 caused a significant decrease in MDA and increased in GST as compared with control +ve group.

Subcutaneous injection of CCl_4 in a dose of (0.1 ml/ 100g BW) induced a significant increase in lipid per oxidation (MDA) and decrease in glutathione transferase (GST). Reactive oxygen species (ROS) are produced as a consequence of normal aerobic metabolism unstable free radical species attack cellular component causing damage to lipids, proteins and DNA .Living organisms have developed complex antioxidant systems to reduce their damage. These antioxidant include enzymes such as lipid peroxidase and glutathione (**Koracevic et al. 2001**).

Malondialdehyde (MDA) (lipid peroxidase) is a lipid degradation product (**Romeo et al.2002**) it is formed by peroxidation of unsaturated fatty acid and is used as a biological marker of oxidative. **Depault et al. (1994)**. Our data indicated that lipid peroxidation concentration in serum showed no significant changes in rats feeding on ration mixed with fennel when compared with c-ve group and disagreement with **Amr (2012) and Mohamad et al. (2011)** who showed the presence of different types of compounds in FSME, such as flavonoids, terpenoids, alkaloids, phenols, and sterols, administration of FSME before irradiation exerted a cytoprotective effect against gamma irradiation, as manifested by a restoration of the MDA level, catalase activity, and GSH content to near-normal levels. In conclusion, FSME may have remarkable anticancer potential against a liver cancer cell.

The obtained results showed that feeding on ration mixed with fennel and tiger nut (5g /100g ration) not induce a significant changes in serum AST, ALT and ALP as compared to control –ve group. Subcutaneous injection of CCl₄at a dose of 0.1ml/100g b. wt. produced a very highly significant increase in serum AST, ALT and ALP (table 5). Feeding on ration mixed with fennel and tiger nut with injected CCl₄ produced a significant decrease in serum AST, ALT and ALP than the group injected CCl₄alone and tended to the–ve control group. These results are in agreement with **Faroket al. (2011) and Amr (2012)**which recorded decreasing in transaminase enzymes. These results correlated with **Hwang (2004)** who indicated that's were not significant different between the control group and tiger nut group.

Fennel did not induce changes of serum total protein, while slightly decreasing when fed on tiger nut. The level of serum albumin when fed animals with plants displayed significantly higher more than control, but induced significant decrease of serum globulin compared with –ve control group. In addition, plants significant produced increase of serum albumin and A: G ratio. (Table,6).These results agreement with **Hwang (2004)**and **Chukwuma et al. (2010)** who indicate thatthere was no significant effect ($P \geq 0.05$) on protein moreover serum albumin level increased significantly in a concentration dependent manner ($p \leq 0.05$). Since total serumproteins and albumin are generally influenced by total protein intake **Onifade and Tewe (1993)**, the obtained results indicate nutritional adequacy of the dietary and the extract proteins. Abnormal serum albumin usually indicates an alteration of normal systemic protein utilization **Apata (1990), Awosanya et al. (1999)** have demonstrated the dependence of blood protein on the quality and quantity of protein source. The reported low level of phytate in the tuber could also have led to the increased absorption of protein from the rat diet. Phytate acts as a chelator, forming proteins and mineral bioavailability **Davies and Gathlin (1991)**. These results disagreement with **Abd**

El-Latif et al.(2001) and Tollba (2003). The level of serum albumin when fed animals with plants displayed significantly higher more than control, but induced significant decrease of serum globulin compared with –ve control group. Feeding on ration mixed with fennel and tiger nut with injected CCl₄ caused a significant increase in total protein, albumin, and globulin and A: G ratio directed toward normal as compared with +ve control group (injected CCl₄). The obtained data are agreement with those reported by **Boonjaraspinyo et al. (2009)**. Noticed that fed fennel have improve effect more than tiger nut (table 6).

While subcutaneous injection of CCl₄ at a dose of 0.1 ml /100 g BW, produced statistically decreased serum protein, albumin, globulin and A: G ratio. The obtained results are in agreement with those reported by **Fouad et al. (1996), Li and Liu (2004)** and **Ogeturk et al. (2004-2005)**. From the above mentioned results, it is easy to notice that CCl₄ clearly affect the liver and its enzymes and this negative effect decrease the liver capacity to synthesis the proteins and also tested plants contain antioxidants and flavonoids which improved nutritional status as anti-inflammatory and beneficial effect on the liver regeneration **Boonjaraspinyo et al. (2009)**. In addition, the results in this study showed significant decreases in total protein, albumin and A : G ratio, which may be due to impaired kidney and liver function. Because albumin is synthesized by the liver, decreased albumin may result from liver damage. It can also result from kidney disease, which allows albumin to escape into the urine(**Kaneko et al. 1997**) and also the major site of synthesis of the plasma proteins is the liver and the second major site is the immune system.

It obvious from table (7) the level of serum urea is not significantly different in animals treated with fennel or tiger nut compared to control. Serum creatinine, however, was higher in tiger nut fed than control animals. These result agreements with **Tollba (2003)** who observed that Creatinine were not affected by adding fennel. Other studies **Abdel-Azeem (2006) and Faroket al. (2011)** approved decreasing in creatinine and uric acid when feeding with fennel and tiger nut.

Rats feeding on ration mixed with each of tested plants with injected serum CCl₄ showed significantly decreased in creatinine and urea concentration than the control group (injected CCl₄ alone) and directed toward the normal control group.

CCl₄ administration resulted in highly significant increase in both serum creatinine and urea, compared to control. There was no significant difference between the concentrations of glucose in the serum of rats given fennel and tiger nut, but it was significantly higher in the serum of rats treated with CCl₄. Rats feeding on ration mixed with tested plants plus CCl₄ showed significantly decreased glucose concentration than control group. These results agreement with **Naglaa et al. (2010) and Hanefi et al. (2004)** because it contains

high amount of many antioxidants which can give chemo-protections against many chemically induced damage in liver and kidney cells.

There was no significant difference between the concentrations of glucose in the serum of rats given fennel and tiger nut table (7). These result agreements with **Sushruta et al. (2006)** and **Hwang (2004)** who investigated there is no significant effect on serum glucose when feeding rats with tested plants.

In results for **Chukwuma et al. (2010)** shows significantly decreased in blood glucose level concentration dependent manner ($p \leq 0.05$) as well as **Vandana et al. (2012)** who show that prolonged treatment with the pet ether fraction of the *Foeniculum vulgare* distillate demonstrated improvement in blood glucose cause HPLC analysis revealed trans-anethole as the bioactive constituent possessing potent aldose reeducates inhibitory action. Trans-anethole could effectively show anti-cataract activity through the increase in soluble lens protein, reduced glutathione, catalase and SOD activity on in vitro incubation of the eye lens with 55 mM glucose.

It is well known that soluble fibers generally increase transit time through the gut, slow emptying of the stomach and slow glucose absorption (**Swaminathan, 2002**). *Cyperus culentus* tubers have high dietary fiber content **Umerie and Enebeli (1997)**, so they may play a major role in lowering blood glucose. Rats feeding tested plants with injected CCl_4 showed significantly decreased in glucose concentration than C+ve. The reported decrease in the maximum insulin- stimulated glucose transport could be a result of the free radical attack on the cell membrane. Free radicals were reported to cause a decrease in the fluidity of the rat liver plasma membrane lipids (**Moustafa et al. 1995**). But glucose concentrations were significantly higher in the serum of rats subcutaneously injected with CCl_4 .

Cholesterol concentration in rats fed on ration mixed with fennel and tiger nut showed a decrease as compared with -ve group while rats injected with CCl_4 produced a very highly significant increase in cholesterol levels as compared with other groups.

Rats fed each of tested plants with injected CCl_4 showed significantly decrease in cholesterol levels directed toward normal as compared with control +ve group table (8). There was no significant difference between the concentration of HDL and LDL in serum of rats feed on ration mixed with fennel and tiger nut (5g/ 100g ration). Serum HDL concentrations were decrease in group injected with CCl_4 when compared with C-ve group. While serum LDL concentrations significant increasing as compared with C-ve group. Groups given each of tested plants with injected CCl_4 showed insignificant increase in HDL and significant decrease in LDL values comparing with the control +ve group. This is shown in table (9).

There was no significant difference between the concentration of triglyceride in serum of rats feed on ration mixed with fennel and tiger nut (5g/ 100g ration)

Serum triglyceride concentrations were increased in group injected with CCl₄ as compared with control C-ve group. While groups given each of tested plants with injected CCl₄ showed a significant decrease in triglyceride values comparing with the control +ve group. This is shown in table (8).

Triglyceride and cholesterol concentrations showed significantly increased in the serum of rats injected with CCl₄. **Abdel-Hamid(2006)** observed the increase in the concentrations of triglyceride and cholesterol in the serum of rats injected with CCl₄. However, the LDL-cholesterol value was significantly higher ($P < 0.01$) in rats groups fed CCl₄ as compared with normal control. This observation due to the CCl₄ absorbed readily from gastrointestinal tract and inhalation via respiratory tract. Cytochrome P450 mediated transfer of an electron to the chlorinated solvents (CCl₄) bound forming free radicals initiates the metabolism of chlorinated solvents. This radical induce lipid peroxidation or attack membrane or lipoprotein; start lipid peroxidation (**Raucy et al. 1993; Grubelet al. 1996**). These results were in agreement with that of **Honma and Suda (1997)** who found that HDL- cholesterol decreased significantly, and marked increase in LDL – cholesterol in serum rats administrated single intraperitoneal dose at 1.0 mg / kg of CCl₄. The author proved that changes in plasma lipoprotein could serve as a sensitive and simple marker for liver disorders caused by chlorinated hydrocarbon solvents such as CCl₄.

This result suggests that cholesterol-lowering activity of the fennel can result from a rapid catabolism of LDL-C through its hepatic receptors for final elimination in the form of bile acids as demonstrated by (**Guimaraes et al. 2000**). **Cao and Prior (1998)** suggested that the hypolipemic activity of fennel could be attributed to the presence of the valuable polyphenolic com-pounds especially tannins, and flavonoids. **Eleni and Bairaktari (2005)** demonstrated that flavonoids and anthocyanins, a heterogeneous group of polyphenols, have exhibited a variety of pharma-cological activities, including the antiatherogenesis effect. Anethole (t-anethole) that is the main com-pond in all fennel volatile oils possesses significant antioxidant activity. The presence of t-anethole and flavonoids content in fennel may be associated with lowering TL, TC, TG and LDL-c levels. So fennel suggested being a new alternative for clinical management of hyperlipidemic patients (**Freire et al. 2005**). Anti-oxidative properties and radical scavenging activity may be the possible mechanisms by which fennel ameliorated the TL, TC, TG and LDL-C. Flavonoids are reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats **Patel et al. (2009)**. Furthermore **Gibney et al. (2002)** reported that hypolipemic effect of fennel may be due to the high content of poly-unsaturated fatty

acids from omega-6 and omega-3 families that found in this plant and these compounds have strong biological properties in low concentrations.

Effect on DNA:

The rats feeding on diet mixed with fennel and tiger nut with injected CCl_4 find out whether fennel and tiger nut protect CCl_4 -induced DNA damage (Fig.1) DNA fragmentation was examined by agarose gel electrophoresis this record in Figure 1 represents the results. Fennel and tiger nut treatment found to be effective (to some extent) to prevent the toxin-induced smear formation suggesting that these substances may possess a protective power for the prevention of liver cells from CCl_4 -induced DNA damage and necrotic death.

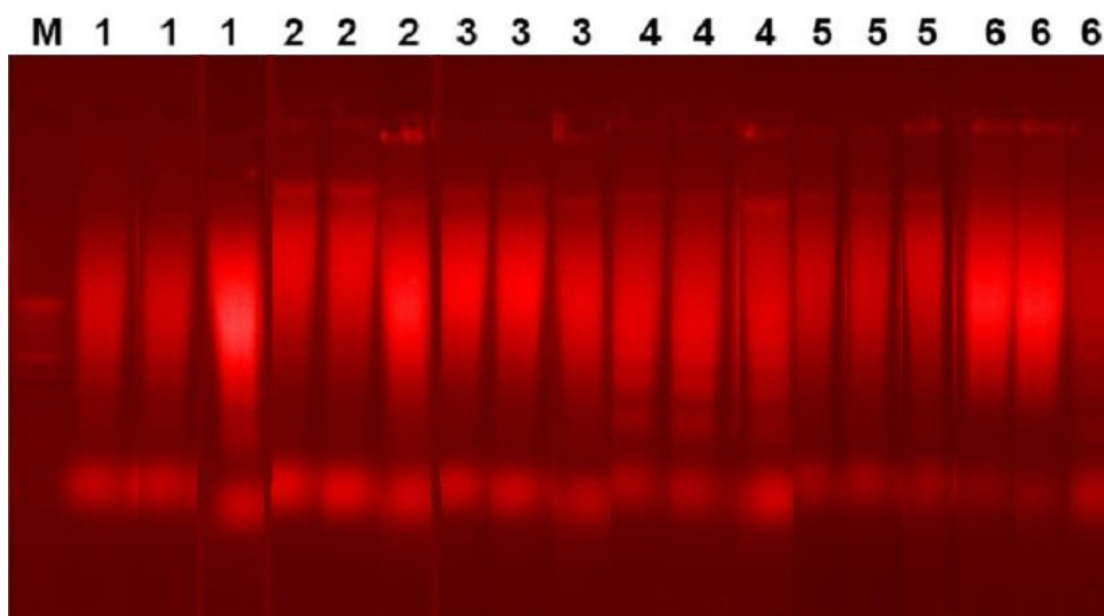


Figure 1: Agarose gel electrophoresis of undigested DNA stained by ethidium bromide. The DNA was extracted from liver samples of :(1) control, (2) CCl_4 treated, (3) fennel fed, (4) tiger nut-fed, (5) fennel fed CCl_4 treated and (6) tiger nut CCl_4 treated rats. (M) 1 kilo base pair marker, 50 bP ladder size.

Our present data illustrated that oxidative stress induced by oxygen-derived species can produce a multiplicity of modifications in DNA including base and sugar lesions, strand breaks, DNA-protein cross-links and base-free sites. If left un-repaired, oxidative DNA damage can lead to detrimental biological consequences in organisms, including cell death, mutations and transformation of cells to malignant cells. In order to find out whether fennel and tiger nut protect CCl_4 -induced DNA damage, DNA fragmentation was examined by agarose gel electrophoresis fennel and tiger nut treatment found to be effective (to some extent) to prevent the toxin-induced smear formation suggesting that these substances

may possess a protective power for the prevention of liver cells from CCl₄-induced DNA damage and necrotic death. At the same time these substances did not induce any fragmentation in DNA when administered alone. Our results are in agreement with **Naglaa et al. (2010)** found that fennel contains many phytochemicals such as thymol, carvacrol, terpinenes, P-thymene and thymol methyl ether, phenolic glycosides, flavonoids, phytosterols-triterp and saponins which have antioxidant and radical scavenging effect, they minimized the amount of reactive oxygen species generated by fatty acid peroxidation and in the case of monenoic fatty acids, the DNA damage can be reduced by a lower lipid peroxidation.

Therefore, according to the present findings, we can conclude that, adding to the diet are more effective as prevention of DNA damage and this may act as a protective agent against diseases such as liver, kidney, diabetes, immune system diseases, and should be focused on plants as fennel and tiger nut with some concentrations and doses suggested for man and possible use as protective agent.

Table 2: Composition of fennel and tiger nut (100 g).

nutrients	% nutrient value	
	Fennel	Tiger nut
Protein	9.5	5.1
Total Fat	10	24.5
Dietary Ash	7	1.69
Carbohydrate	42.3	43.28
Fiber	18.5	8.85

Table 3: Feed intake and body weight gain of control and experimental animals

Groups	B. Wt. gain/g	Feed intake (g/ period)
Gr1 (-ve)	74.4 ± 4.87 ^c	2050
Gr2(+ve CCl₄)	48.1 ± 3.17 ^e	1870
Gr3(fennel)	82.531 ± 8.37 ^b	2145
Gr4(tiger nut)	90.3 ± 7.41 ^a	2970
Gr5(fennel+CCl₄)	67.19 ± 5.31 ^d	2185
Gr6(tiger+CCl₄)	69.23 ± 6.51 ^d	2170

a, b, c, d insignificantly different between two comparison groups within the same litter and column using Duncan Range Multiple test at $P \leq 0.05$.

Table 4: Mean glutathione S-transferase activity and MDA level in control and experimental rats (n=5).

Groups	Gluthathione-S_ Transferase (U/ml)	MDA (mmol/ml)
Gr1(-ve)	14.7±0.78 ^d	4.679±0.156 ^c
Gr2(+ve CCl ₄)	10.368±0.34 ^e	6.26 ±0.49 ^a
Gr3(fennel)	32.52 ±3.36 ^a	5.19±0.016 ^b
Gr4(tiger nut)	27.13±2.01 ^b	4.95±0.043 ^c
Gr5(fennel+CCl ₄)	28.31± 2.43 ^b	5.27±0.019 ^b
Gr6(tiger+CCl ₄)	23.17±2.29 ^c	4.88±0.068 ^c

insignificantly different between two comparison groups within the same litter and column using Duncan Range Multiple test at $P \leq 0.05$.

Table 5: Mean activities of serum transaminases and Alkaline Phosphatase in control and experimental rats (n=5).

Groups	ALT(u/l)	AST(u/l)	AP(u/l)
Gr1 (-ve)	13.418±0.53 ^c	29.63±0.365 ^c	40.58 ±0.756 ^b
Gr2(+ve CCl ₄)	30.51±1.39 ^a	55.08±5.85 ^a	80.91±3.142 ^a
Gr3(fennel)	13.25±1.245 ^c	27.38±4.299 ^c	39.88±1.86 ^b
Gr4(tiger nut)	12.50±0.738 ^c	29.18±5.659 ^c	38.91±2.09 ^b
Gr5(fennel+CCl ₄)	20.08±2.462 ^a	39.52±6.024 ^b	42.31±3.15 ^b
Gr6(tiger+CCl ₄)	15.082 ±3.52 ^{bc}	41.42±6.079 ^b	36.92±1.99 ^c

a, b, c, d insignificantly different between two comparison groups within the same litter and column using Duncan Range Multiple test at $P \leq 0.05$.

Table 6: Mean values of total serum protein, albumin, globulin, and A/G rats (n=5).

Groups	T. protein(g/dl)	albumin(g/dl)	globulin(g/dl)	Ratio(A/G)
Gr1(-ve)	7.4860±0.38 ^a	3.782±0.03 ^b	3.54±0.279 ^b	1.09±0.081 ^b
Gr2(+ve CCl ₄)	5.674±0.179 ^c	2.758±0.22 ^c	2.916 ±0.16 ^a	0.097±0.017 ^a
Gr3(fennel)	7.3±0.189 ^a	4.563±0.09 ^a	2.73±0.146 ^a	1.67±0.0558 ^d
Gr4(tiger nut)	7.0380±0.24 ^b	4.25±0.112 ^a	2.788 ±0.15 ^a	1.462±0.07 ^d
Gr5 (fennel+CCl ₄)	7.0656±0.20 ^a	3.818±0.87 ^b	3.248±0.24 ^b	1.18±0.109 ^c
Gr6 (tiger+CCl ₄)	6.94 ±0.172 ^b	3.36±0.05 ^{bc}	3.578±0.84 ^c	0.94 ±0.051 ^b

a,b,c,d, insignificantly different between two comparison groups within the same litter and column using Duncan Range Multiple test at $P \leq 0.05$.

Table 7: Serum Creatinine, urea and Glucose in control and experimental rats (n=5)

Groups	Creatinine(mg/dl)	Urea (mg/dl)	Glucose(mg/dl)
Gr1(-ve)	0.762±0.08 ^d	16.50±0.259 ^d	89.77 ±0.7 ^c
Gr2(+ve CCl ₄)	1.236±0.06 ^a	77.89±1.53 ^a	154.67 ±2.1 ^a
Gr3(fennel)	0.791±0.03 ^{cd}	17.3±0.9 ^d	87.93±1.78 ^c
Gr4(tiger nut)	0.81±0.005 ^c	18.03±1.2 ^d	85.37 ±2.1 ^c
Gr5(fennel+CCl ₄)	0.83±0.03 ^c	42.34±4.5 ^c	112.27±2.9 ^b
Gr6(tiger+CCl ₄)	0.89±0.02 ^b	51.62±4.3 ^b	116.19±3.76 ^b

a, b, c, d insignificantly different between two comparison groups within the same litter and column using Duncan Range Multiple test at $P \leq 0.05$.

Table 8: Serum cholesterol, HDL, LDL and triglycerides in control and experimental rats (n=5).

Groups	Total cholesterol (mg/dl)	HDL (mg/dl)	Triglyceride (mmol/5ml)	LDL(mg/dl)
Gr1 (-ve)	119.49±2.985 ^c	61.2±0.3 ^a	25.515±4.66 ^c	17.0±0.9 ^c
Gr2 (+ve CCl ₄)	198.8±5.62 ^a	48.77±0.6 ^b	49.075 ±8.44 ^a	28.1±0.93 ^a
Gr3(fennel)	104.197±4.07 ^d	59.448±2.876 ^a	28.3±1.635 ^c	16.23±1.09 ^c
Gr4(tiger nut)	106.19 ±3.16 ^d	61.768 ±6.49 ^a	26.294 ±2.025 ^c	15.98±0.98 ^c
Gr5 (fennel+CCl ₄)	140.08±9.013 ^b	50.48±2.96 ^b	41.508 ±8.1 ^b	23.6 ±1.62 ^b
Gr6 (tiger+CCl ₄)	134.433 ±6.16 ^b	49.60±3.758 ^b	40.785 ±2.825 ^b	22.79±1.02 ^b

a, b, c, d insignificantly different between two comparison groups within the same litter and column using Duncan Range Multiple test at P ≤0.05.

References

- **Abdel-Azeem, F. (2006):** Effect of using fenugreek and fennel seeds as natural feed additives on performance of broiler chicks. Egyptian Journal of Nutrition and Feeds; 9(2):277-297. 48.
- **Abdel – Hamid, N. M. (2006):** Diphenyl Dimethyl bicarboxylate as an Effective treatment for chemical-induced fatty liver in rats. Afr. J. Biomed. Res., 9: 77 – 81.
- **Abd El-Latif, S. A.; Faten, A. I. and Elkiaty, A. M. (2001):** Effect of feeding dietary Thyme, Black cumin, Dianthus, and Fennel on productive and some metabolic responses of growing Japanese quail. Biotechnology in Animal Husbandry, 17(5/6):191-196.
- **Amr A. Rezq (2012):** Beneficial Health Effects of Fennel Seeds (Shamar) on Male Rats Feeding High Fat-Diet Med. J. Cairo Univ., Vol. 80, No. 2, March: 101-113.

- **Aneja, R.; Upadhyaya, G.; Prakash, S.; Dass, S. K. and Chandra, R. (2005):** Ameliorating effect of phytoestrogens on CCl₄ – induced oxidative stress in the livers of male wistar rats. *Artif. Cells Blood Substit. Immobil. Biotechnol.*, 33(2): 201 – 203.
- **A.O.A.C. (1995):** Official Methods of Analysis of the Association of Analytical chemists, 16 ed. P. 936. 16. Washington U.S.A.
- **Apata, D. F. (1990):** Biochemical, Nutritional and Toxicological Assessment of some Tropical Legume seeds. Ph.D. Thesis, University of Ibadan, Nigeria.
- **Awosanya, B.; Joseph, J. R.; Apata, D. F. and Agbola, M. A. (1999):** Performance, blood chemistry and carcass quality attributes of rabbits fed raw and processed pueraria seed meal. *Trop. J. Anim. Sci.*, 2: 89-96.
- **Bamishaiye E. I.; Muhammad, N. O. and Bamishaiye O. M. (2010):** Assessment of biological value of tiger nut (*Cyperus Esculentus*) tuber oil meal-based diet in rats. Scholars Research Library. *Annals of Biological Research*, 1 (4): 274-280.
- **Borah, R. C.; Mohan, P.; Choudhury, B. H. and Barua, I. C. (2004):** Hepatoprotective activity of *Phyllanthus fraternus L.* and *Glycosmis pentaphylla (Rety) correa* used against jaundice in N.E. India. Bioprospecting of commercially important plants . Proceedings of the national symposium on "Biochemical approaches for utilization and exploitation of commercially important plants"; J. orhat, India. 259- 262.
- **Boonjaraspinyo, S.; Boonmars, T.; Aromdee, G.; Srisawangwong, T.; Kaewsamut, B. and Pinlaor, S. (2009):** Turmeric reduces inflammatory cells in hamster opisthorchiasis. *Parasitol. Res.*, 105(5): 1459-63.
- **Cao, G. and Prior R. L. (1998) :** Comparison of different ana-lytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry*, 44: 1309-1315.
- **Chukwuma, E. R.; Obioma, N. and Christopher, O. I. (2010):** The phytochemical composition and some biochemical effects of Nigerian tiger nut (*Cyperuses culentus, L.*) tuber. *Pakistan Journal of Nutrition*; 9(7):709-715.
- **Coskuner, Y.; Ercan, R, Karababa, E. and Nazlican, A. N. (2002):** Physical and chemical properties of chufa (*Cyperusesculentus L*) tubers grown in the C, ukurova region of Turkey. *J. Sci. Food Agri.*, 82:625–31.
- **Davies, D. A. and Gathlin, D. M. (1991):** Dietary mineral requirement of fish and shrimp. Akiyama, D.M. and R. Tan (Eds.). Proceedings of the aquaculture feed processing and nutrition workshop, Thailand and Indonesia, 19-25 September, American soybean association, Singapore, pp: 49-67.
- **Depault, A. C.; Torreilles, J. and Cristel, J. P. (1994):** Membrane lipids as a preferential target for oxidative process. In paoletti R.; Saunlesson, B. Catapan, A.L.; Poli, A. and Rinetti, M.(Eds) oxidative processes and antioxidants. Raveb press, Ltd, New york PP.73-96.

- De Vries, F. T. (1991):** Chufa (*Cyperus esculentus*, Cyperaceae): Weedy Cultivar or a Cultivated Weed? *Econ. Bot.* 45: 27-37.
- Eleni, T. and Bairaktari, P. (2005):** Evaluation of methods for the measurement of low-density lipoprotein cholesterol. *J. of Cardio. Pharmacol. and Therap.*, 10: 45-54.
- Egyptian Pharmacopeia (1984):** Cairo general Organization for Government printing office .3thed (1) 802.
- Farok, M. A.; Atiat, M. E.; Usama, E. M.; Yasser, M. E.; and Rasha, H. H. (2011):** studies of effect of using saffron , cyperus , honebee and their combination on rats suffering from hyperlipidemia (II) The 6th Arab and 3rd International Annual Scientific Conference
- Faulkner, W. P. and King, J. W. (1976):** Fundamentals of clinical chemistry 2nded. pp. 994-998. Saunders Company, Philadelphia, USA.
- **Ferguson, LR (1994):** Review antimutagens as cancer chemopreventive agents in the diet. *Mutat. Res.*, 307:395-410.
 - **Fouad, F.M.; Mamer, O. A. and Shahidi, F. (1996):** Acute-phase response in rat to carbon tetrachloride-azathioprine induced cirrhosis and partial hepatectomy of cirrhotic liver. *J. Toxicol. Environ. Health.*, 47(6) : 601-615.
 - **Fredrickson, D. S.; Levi, R. L. and Less, R. S. (1967):** Determination of cholesterol .N. *Engl. J. Med.*, 276: 148-156.
 - **Freire, R. S.; Morais, S. M.; Catunda, F.; and Pin-heiro, D. (2005):** Synthesis and antioxidant, anti-inflammatory and gastroprotector activities of anethole and related compounds. *Bioorg. Med. Chem.*, 13:4353-4358.
 - **Friedewald, W. T.; Levy, R. I. and Fredrickson, D.S. (1972):** Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *CLin. Chem.* 18 (6) 499-505.
 - **Gibney, M. J.; Vorster, H. H. and Kok, F. J. (2002):** Introduction to Human Nutrition, Blackwell 298 Science, Oxford.
 - **Gruebele, A.; Zawaski, K.; Kaplan, D. and Novak, R. F. (1996):** Cytochrome P4502E1- and cytochrome P4502B1/2B2-catalysed carbon tetrachloride metabolism, *Drug Metab. Dispos.*, 24: 15-22.
 - **Guimaraes, P.R.; Galvao, A.M.; Batista, C.M.; Azevedo, G.S.; Oliveira, R.D.; Lamounier, R.P.; Freire, N.; Barros, A.M.; Sakurai, E.; Oliveira, J.P.; Vieira, E.C. and Alvarez-Leite, J. I. (2000):** Eggplant (*Solanum melangena*) infusion has a modest and transitory effect on hypercholesterolemic subjects. *Brazilian Journal Medical Biological Research*, 33: 1027-1036.

- **Habig, W. and Pabst, M.J. (1974)** : Glutathion –S- Transferases the first enzymatic step in mercapturic acid formation. Biol-chem., 249: 7130-7139.
- **Hanefi, Ö.; Serdar, U.; Irfan, B.; Ismail, U.; Ender, E.; Abdurrahman, Ö. and Zübeyir, H.(2004)**: Hepatoprotective effect of *Foeniculumvulgare* essential oil:A carbon-tetrachloride induced liver fibrosis model in rats. Scand. J. Lab. Anim. Sci. No. 1, Vol. 31.
- **Honma, T. and Suda, M. (1997)**: Changes in plasma lipoproteins as toxicity markers for carbon tetrachloride, chloroform, and dichloromethane, Ind. Health., 35: 519-531.
- **Horwitz, W. (1980)**: Official methods of analysis. (13th edition) Benjamin prouklin St. Washington D. C.
- **Hwang, E. (2004)**: The effect of feeding of tubers of *Cyperusesculentus* on growth and hematological changes in rats. Journal of Veterinary Clinics. 21(3):259-263. 23 ref.
- **Ibrahim, F. A.; Ayad, K. M. K.; Ahmed, M. E. and El-Kholeny, M. E.(2013)**: Effect of using Chufa tubers (*Cyprus esculentus* L.) in Zaraibigoats diets on the resultant milk and labenh.Egyptian Journal of Sheep and Goat Sciences; 8(1):201. 37 ref.
- **Jaramillo-Juarez, F.; Maria Luisa, R. V.; Ana Rosa, R. S.; Maria Consolacion, M.; Ganaro, G. o.; Javier, L.; Francisco, A. P.; Jose, L. R.(2008)**: Acute renal failure induced by carbon tetrachloride in rats with hepatic cirrhosis .Ann.Hepatal .Oct-Dec 7(4):331-8 .
- **Kaneko, J.J; Harvey, W. J. and Bruss, L.M.(1997)**: Clinical Biochemistry of Domestic Animals. 5thed., Academic Press, San Diego, London.
- **Knight, T. A.; Anderson, S. and James, M. R. (1972)**: Chemical basis of the sulphovenilim reaction for estimating total serum lipid. J. Clinc. Chem., 18:199.
- **Koracevic, D.; Koracevic, G. and El-Aaser, A. A. (2001)**: J. Clin. Path., 54: 356-361.
- **Lee, H.S.; Keum, K.Y. and Ku, S.K.(2007)**: Effects of Picrorrhizarizoma water extracts on the subacute liver damages induced by carbon tetrachloride. J. Med. Food., 10(1) : 110-117.
- **Less, R. (1975)**: Aflatoxin determination and T.L.C.Examination food analysis, 3rd published by Leonard, H. u. books, p. so.
- **Li , Y ; Li, Y. and Liu, G. T. (2004)**: Protective effects of bicyclol on liver fibrosis induced by carbon tetrachloride. Yi. Xue. Za. Zhi ., 84 (24): 2096-2101.
- **Lippman, S.M.; Benner, S.E. and Hong, W.K. (1994)**: Cancer Chemoprevention. J. Clin. Oncol., 12 :851-873.
- **Manzoor, A. R.; Bilal, A. D., Shahnawaz, N. S.; Bilal, A. B. and Mushtaq. A. Q. (2012)**: *Foeniculum vulgare*: Apprehensive review of its traditional use, phytochemistry, pharmacology, and safety, Arabian Journal of Chemistry.
- **Mc Gregor, D. and Lang, M. (1996)**: Carbon tetrachloride: genetic effects and other modes of action. Mutat. Res., 366: 181-195.
- **Mohamad, R. H.; El-Bastawesy, A. M.; Abdel-Monem, M. G.; Noor, A. M.; Al-Mehdar, H. A. R.; Sharawy, S. M. and El-Merzabani, M. M.(2011)**: Antioxidant and

- anticarcinogenic effects of methanolic extract and volatile oil of fennel seeds (*Foeniculum vulgare*). Journal of Medicinal Food; 14(9):986-1001. 60.
- **Moustafa S.A. Webster J.E. Mattar F.E. (1995):** Effects of Aging and Antioxidants on Glucose Transport in Rat Adipocytes Gerontology 1995;41: 301–307 .
 - **Naglaa, M. E.; Halima, S. A.; Hoda, F. B.; Sherifa, H. S.; Ekram, S. A. and Fahmy, K. H. (2010):** Antimutagenic and Chemoprevention Potentialities of Sweet Fennel (*Foeniculumvulgare, Mill.*) hot water crude extract. Journal of American Science; 6(9):831-842.
 - National cholesterol Education Program (N.C.E.P.) Recommendation for** Measurement of high –Density Lipoprotein cholesterol: Executive summary. Clin Chem. 1995: 1427-1433.
 - **Oderinde, R.A.; and Tairu,O.A. (1988):**Evaluation of the properties of yellow nutsedge(*Cyperusesculentus*) tuber oil. J. Agric. Food Chem., 28, 233-237.
 - **Ohkawa,H; Ohishi,W. and Yagi, K.(1979) :** Assay for lipid peroxides in animal tissues by thiobarbitutric acid reaction. Anal. Biochem., 95:351.
 - **Ogeturk, M. ;Kus, I. ; Kavakli, A. ; Zararsiz, I. ; Ilhan, N. and Sarsilmaz, M. (2004):** Effects of melatonin on carbon tetrachloride – induced changes in rat serum. J. Physiol. Biochem., 60(3): 205 – 210.
 - **Ogeturk, M.;Kus, I.; Colakoglu, N.; Zararsiz, I.; Ilhan, N. and Sarsilmaz, M. (2005):**Caffeic acid phenelthyl ester protects kidneys against carbon tetrachloride toxicity in rats. Journal Ethnopharmacology., 97(2): 273 – 280.
 - **Qiu, D. K.; Hua, J.; Li, J. Q. and Li, E. L. (2005):**CD14 expression on Kupffer cells during the course of carbon tetrachloride-mediated liver injury. Chin. J. Dig. Dis.; 6(3):137-41.
 - **Onifade, A. A. and Tewe, O. O. (1993):** Alternative tropical energy feed performance in rabbit diets: Growth performance, diet digestibility and blood composition. World Rabbit Sci., 1: 17.
 - **Patel, D. K.; Patel, K. A.; Patel, U. K.; Thounaoja, M. C.; Jadeja, R. N.; Ansarullah, G.S.; Salunke, S.P.; Devkar, R.V.and Ramachandra, A.V (2009):** Assessment of lipid lowering effect of Sidarhomboidea. Roxbmethanolic extract in experimentally induced hyperlipidemia. J. Young Pharma. 1 (3): 233-238.
 - **Raucy, J. L.; Kraner, J. C. and Lasker, J. M. (1993):**Bioactivation of halogenated hydrocarbons by cytochrome P4502E1, Crit. Rev. Toxicol., 23: 1-20.
 - **Reitman, S. and Frankel, S. (1957):** Acolorimetric determination of serum glutamic oxaloacetic and glutamic pyruic transaminase. Am. J. Clin. Path., 28: 56-58.
 - **Rita, E. S. (2009):** The use of tiger-nut (*Cyperusesculentus*), cow milk and their composite as substrates for yoghurt production. Pak. J. Nutr., 6: 755-758.

- **Romeo, M.; Bsnnani, N.; Gnassia, B. M.; Lafavire, M.; Rydberg, P. and Luning, B. (2002):** Application of a modified edman procedure for measurement of protein adducts P: Mechanisms of formation and degradation of phenyl thiohydantoins. *Chemical Research in Toxicology*, 15:570-581.
- **Roy, A.V. (1970):** a rapid method for alkaline phosphates estimation *clin. chem.*, 16: 431.
- **Sellins, K. S. and Cohen, J. J. (1987):** Gene induction by gamma-irradiation leads to DNA fragmentation in lymphocytes. *J. Immunol*; 139:3199–206.
- **Snedecor, G. W. (1969):** Statistical methods "Fourth Ed.; The Iowa state, college press, Ames Iowa.
- **Simeonova, P. P.; Gallucci, R. M.; Hulderman, T.; Wilson Kommineni, C.; Rao, M. and Luster, M. I. (2001):** The role of tumor necrosis factor- α in live toxicity, inflammation, and fibrosis induced by carbon tetrachloride, *Toxicol. Appl. Pharmacol.*, 1; 177: 112-120.
- **SPSS 14 (2006):** "Statistical Package for Social Science, SPSS for windows Release 14.0.0, 12 June, 2006." Standard Version, Copyright SPSS Inc., 1989-2006, All Rights Reserved, Copyright SPSS Inc.
- **Sonnenwirth, A. and Jaret, L. (1980):** Grad wholes clinical laboratory methods and diagnosis. vol. 18th ed Mosby. London.
- **Sripanidkulchai, B.; Tattawasart, U.; Laupatarakasem, P.; Vinitketkumneur, U.; Sripanidkulchai, K.; Furihata, C. and Matsushima T (2002):** Antimutagenic and anticarcinogenic effects of *Phyllanthus amarus*. *Phyto. Med.* 9: 26-32.
- **Sushruta, K.; Satyanarayana, S.; Srinivas, N. and Sekhar, J. R. (2006):** Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected umbelliferous fruits used in culinary practices. *Tropical Journal of Pharmaceutical Research*; 5(2):613-617. 22.
- **Swaminathan, M., (2002):** Essentials of Food and Nutrition. Volume 1. The Bangalore Printing and Publishing co .Ltd.
- **Tollba, A. A. H. (2003):** Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions. 1 - Thyme (*Thymus vulgaris L.*) or fennel (*Foeniculum vulgare L.*). *Egyptian Poultry Science Journal*; 23(2):313-326. 25.
- **Trinder, P. (1969):** Enzymatic Determination of Glucose .*Ann. Clin. Biochem.*, 6 : 26.
- **Umerie, S.C. and J.N.C. Enebeli, (1997):** Malt caramel from the nuts of *Cyperus esculentus*. *J. Biol. Resource Technol.*, 8: 215-216.
- **Vandana, D.; Chaitanya, K.; Manish, K.; Chandrakant, M.; Vivek, H. and Akalpita, A. (2012):** Inhibition of aldose reductase and anti-cataract action of trans-anethole isolated from *Foeniculum vulgare Mill.* fruits. *Food Chemistry*; 132(1):385-390. 32.
- **Wattemberg, L.W. (1985):** Chemoprevention of cancer. *Cancer Res* 45:1-8.

- **Wong, B. H.; Zuzel, K. A.; Rahman, K. and Billington, D. (1998):** Protective effect of aged garlic extract against bromobenzenetoxicity to precision cut rat slices. Toxicology. 126(3): 213-222.
- **Young, D. S. (1995):** Effect of drug on clinical laboratory tests 4thEd .P.3-16 to 3-22.
- **Youssef, A. S. (2000):** Comparative histopathological and immunohistochemical studies on the hepatoprotective activity of garlic and curcumin. Egyp. J. Comp. Path. And Clinic. Path., 13(2): 1-11.