



Effect of some plant oils on some oxidant – antioxidant parameters in alloxan – induced diabetic male rats

Khalid G. Al-Fartosi ^{1*}, Alyaa Majid², Mohammed A. Auda ²

¹ Department of Biology, College of Science, university of Thi-Qar, Iraq

² Department of Chemistry, College of Science, University of Thi-Qar, Iraq

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***Corresponding author:**

Email address:

khalidalfartosi@yahoo.com

Abstract

The present study aimed to evaluate the influence of plant oils pellet containing of black seed (10%), cinnamon oil (10%),

olive oil (10%) and ginger oil (10%) on some oxidant – antioxidant parameters of male rats, these parameters included glucose, malondialdehyde, iron, transferrin, ceruloplasmin and albumin. Eighteen male rats divided into three groups, the first group was control intraperitoneally received 0.5 ml saline solution, fed ad libitum on normal commercial chow and had free access to water, the second group was diabetes rats fed with the same diet given in group (1) and the third group was diabetes animals fed with plant oils pellet containing black seed oil 10%, cinnamon oil, 10% olive oil 10%, ginger oil 10% respectively, daily for 5 weeks. The results indicated that alloxan and normal diet caused a significant increase in serum glucose, malondialdehyde, iron and ceruloplasmin, while the level of transferrin and albumin was a significant decreased in diabetic animal group compared with control group. The male rats were fed with plant oils pellet show a significant decrease in the level of serum glucose, malondialdehyde, iron, ceruloplasmin, while a significant increase in the level of serum transferrin and albumin in comparison to diabetic animal group.

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Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin that frequently results in severe metabolic imbalances and pathological changes in many tissues (Maritim *et al.*, 2003). Dysfunction of the gastrointestinal tract is common among diabetic patients (Zhao *et al.*,

2006). As many as 75% of patients visiting diabetes clinics report significant gastrointestinal symptoms (Folwaczny *et al.*, 1999). The intestinal mucosa is vulnerable to oxidative stress on account of the constant exposure to reactive oxygen species (ROS) generated by several conditions such as ischemia/ reperfusion, inflammatory bowel disease, surgical stress, and diabetes (Bhor *et al.*, 2004). Increased oxidative stress is important in the development and progression of diabetes and related complications.

The protective effects of exogenously administered antioxidants have been extensively studied in animal models in recent years. Several studies have shown that consumption of antioxidant vitamin and nutrient rich antioxidant such as ginger decrease diabetic complications and improve the antioxidant system of the body (Bianca *et al.*, 2000). Recent experimental studies have shown the therapeutic effects Black seed oil on diabetic animals (El-Dakhakhny *et al.*, 2002; Alsaif, 2008). However its effects on blood glucose in human subjects are of an interest. Although Najmi *et al.*, (2008) had shown therapeutic effects Black seed oil on the metabolic syndrome including the blood glucose.

True cinnamon (*C. zeylanicum*) is among 300 species of *Cinnamomum* that belong to the Lauraceae family. The aromatic bark of the cinnamon tree is used worldwide for culinary purposes, but it is also used in Ayurvedic and traditional Chinese medicine for its hypoglycemic, digestive, antispasmodic, and antiseptic properties (Battaglia 1995). Animal studies have demonstrated that cinnamon, and its active constituent cinnamaldehyde, doses dependently improve glycaemic control and hyperlipidemia in normal and streptozotocin-induced diabetic rats (Kannappan *et al.*, 2006; Kim *et al.*, 2006; Subash Babu *et al.*, 2006). Recently, Kwon and coworkers described that cinnamon oil protects from the streptozotocin-induced β -cell damage in vivo and in vitro and proposed inhibition of iNOS protein expression -mediated at the transcriptional level through the inhibition of NF- κ B activation and iNOS transcription- as a plausible mechanism underlying this effect (Kwon *et al.*, 2006). Human's clinical studies on low fat diets also show reversal of the disease. Many studies have shown low-fat diets to be effective in controlling diabetes (Barnad *et al.*, 1983). Olive oil is considered as the pillar of the Mediterranean diet, since it improves the major risk factors for cardiovascular disease, such as the lipoprotein profile, blood pressure, glucose metabolism and antithrombotic profile. Endothelial function, inflammation and oxidative stress are also positively modulated. Some of these effects are attributed beside the monounsaturated fatty acids (MUFA) to the minor components of virgin olive oil (Al-Jamal and Ibrahim, 2001). Hydrocarbons, polyphenols, tocopherols, sterols, triterpenoids and other components, despite their low concentration, non-fatty acid constituents may be of importance because studies comparing monounsaturated dietary oils have reported different effects on cardiovascular disease. Most of these compounds have demonstrated antioxidant, anti-inflammatory and hypolipidemic properties (Perona *et al.*, 2006). Moreover, MUFA-rich diet prevents central fat redistribution and the postprandial decrease in peripheral adiponectin gene expression and insulin resistance induced by a carbohydrate-rich diet in insulin-resistant subjects (Paniagna *et al.*, 2007). Several studies have reported that antioxidants and vitamin A, B, C, and E in diet can protect sperm DNA from free radicals and increase blood testis barrier stability (Wolff *et al.*, 1991; Baynes and Thorpe, 1999). Nowadays ginger rhizome (*Zingiber officinale* R., family: *Zingiberaceae*), is used worldwide as a spice. Both antioxidative (Palmeria *et al.*, 2001) and androgenic activity of *Z. officinale* were reported in animal models. All

major active ingredients of *Z. officinale*, such as *Zingerone*, *Gingerdiol*, *Zingibrene*, and *gingerols shogaols*, have antioxidant activity (Sexton and Jarow, 1997). Besides, other researches showed that ginger oil has dominative protective effect on DNA damage induced by H_2O_2 and might act as a scavenger of oxygen radical and might be used as an antioxidant (Peluso, 2006). Therefore, the present study was carried out to assess the effects of these oils on some oxidant-antioxidants parameters in alloxan-induced diabetic male rats.

Materials and Methods

Animals

Eighteen male albino rats of the *Rattus norvegicus*, weighing (130-150 g) were included in the present study. The experimental animals were obtained from the animal house of Biology Department / College of Science, Thi-Qar University/ Iraq. The experimental animals housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature ($21 \pm 1^\circ C$) and 12:12 h light-dark cycle. Wister rats were fed *ad libitum* on normal commercial chow and had free access to water. The animals divided into three groups: -

- Group (1) were served as control, intraperitoneally received 0.5 mL saline solution, fed *ad libitum* on normal commercial chow and had free access to water.
- Group (2), diabetic rats were fed with the same diet given in group 1.
- Group (3), diabetic rats were fed with plant oils pellet containing black seed (10%), cinnamon oil (10%), olive oil (10%), ginger oil (10%) respectively, daily for 5 weeks.

Diabetes Induction

After fasting of 18 hours, the rats were intraperitoneally injected with alloxan (BDH, England) at a single dose of 125 mg/kg (body weight) in 1 ml saline solution. After injection, the rats had free access to food and water. Diabetes was allowed to develop and stabilize in these alloxan-treated rats over a period of seven days. Diabetes mellitus was defined in these rats using determination of fasting blood glucose levels. The rats showing fasting blood glucose more than 200 mg/dl were considered diabetic and selected for the experimentation.

Biochemical Measurement

At the end of 5 weeks, the experimental animals were fasted for 12 hours, water was not restricted, and then blood samples were drawn from diethyl ether anaesthetized rats. Serum was obtained after the blood was allowed to clot at room temperature and centrifuged at 3000 rpm for 15 minutes. Sera were then collected and stored in freezer till the determination time of the levels of glucose, malondialdehyde (MDA), iron (Fe), transferrin (Tf), ceruloplasmin (Cp), albumin(Alb).

Statistical Analysis

Data were statistically analyzed using Package Social Sciences (SPSS) for Windows version 12.0 software. All experimental data were expressed as mean \pm standard deviation(SD). Statistical analysis was performed by the least significance difference (LSD) method. The $p < 0.01$ level of probability was used as the criteria of significance.

Results

Table (1) explained the effect of some plant oils on some oxidant-antioxidant parameters of diabetic male rats. The results indicated a significant increase ($p < 0.01$) in concentrations of serum glucose in diabetic male rats group (2) in comparison with control group (1), while there was a significant decrease ($p < 0.01$) in concentrations of serum glucose in diabetic male rats group(3) were fed with plant oils pellet in comparison with diabetic male rats group (3) daily for 5 weeks, figure (1). There was a significant increase ($p < 0.01$) in concentration of serum malondialdehyde in group (2) in comparison with control group (1), whereas there was a significant decrease ($p < 0.01$) in concentration of serum malondialdehyde in group (3) in comparison with group (2), figure (2).

Table.1: shows the effect of some plant oils on some oxidant-antioxidant in alloxan – induced diabetic rats. Each value represents mean \pm SD values with non-identical superscript (a , b or c...etc) were considered significantly differences ($P < 0.01$).

Parameters	Group (1)	Group (2)	Group (3)	LSD
Glucose (mg/dl)	94.38 \pm 4.99 ^b	224.78 \pm 24.35 ^a	101.59 \pm 4.76 ^b	47.12
MDA(nmol/ml)	13.24 \pm 1.30 ^c	44.23 \pm 3.85 ^a	26.98 \pm 2.35 ^b	10.48
Iron (μ mol/L)	20.53 \pm 1.46 ^c	49.33 \pm 3.95 ^a	31.47 \pm 0.77 ^b	1.84
Transferrin(g/L)	3.17 \pm 0.13 ^a	1.47 \pm 0.49 ^b	2.9 \pm 0.36 ^a	8.46
Ceruloplasmin (g/L)	5.68 \pm 0.53 ^b	7.63 \pm 0.15 ^a	5.44 \pm 0.31 ^b	1.43
Albumin(g/L)	50.15 \pm 0.81 ^a	41.89 \pm 1.02 ^b	49.56 \pm 0.72 ^a	3.70

- Group (1) = Control rats received 0.5 ml saline solution, fed ad libitum on normal commercial chow and had free access to water
- Group (2) = Diabetes rats were fed with the same diet given in group 1.
- Group (3) = Diabetes animals were fed with plant oils pellet containing 10% black seed oil,10% cinnamon,10% olive oil,10% ginger oil respectively. Daily for 5 weeks.

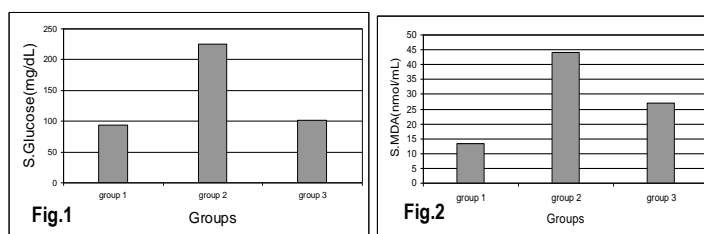


Figure.1: Shows the serum glucose levels in experimental groups.

Figure.2: Shows the serum MDA levels in experimental groups.

The results showed significant increase ($p < 0.01$) in concentration of serum iron in group (2) in comparison with control group (1), while there was a significant decrease ($p < 0.01$) in concentration of serum iron in group(3) in comparison with group (2), figure (3). The results indicated a significant decrease ($p < 0.01$) in concentration of serum transferrin in group (2) in comparison with control group (1), while there was a significant

increase ($p < 0.01$) in concentration of serum transferrin in group (3) in comparison with group (3), figure (4).

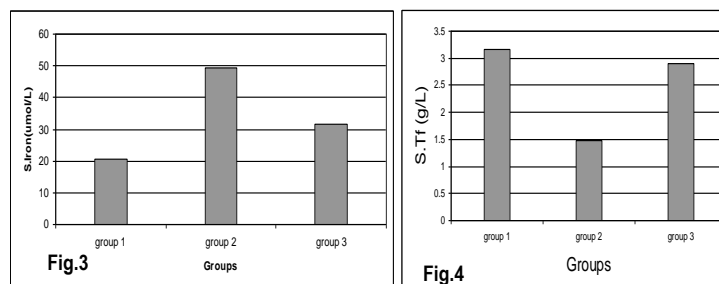
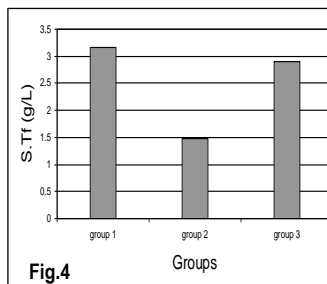


Figure.3: Shows serum Iron levels in experimental groups.



Figur.4: Shows serum Transferrin levels in experimental groups.

The results showed significant increase ($p < 0.01$) in concentrations of serum ceruloplasmin in group (2) in comparison with control group (1), while there was a significant decrease ($p < 0.01$) in concentration of serum ceruloplasmin in group (3) in comparison with group (2), figure (5). The results indicated a significant decrease ($p < 0.01$) in concentrations of serum albumin in group (2) in comparison with control group (1), while there was a significant increase ($p < 0.01$) in concentrations of serum albumin in group (3) in comparison with group (3), figure (6).

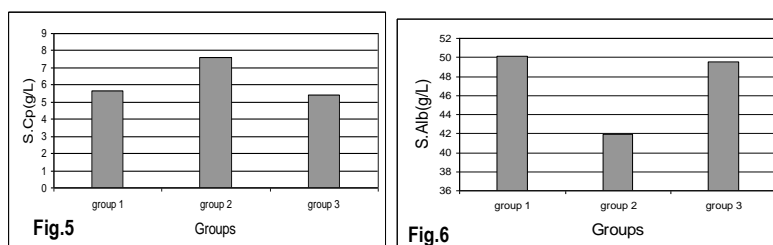


Figure.5: Shows serum Ceruloplasmin levels in experimental groups.

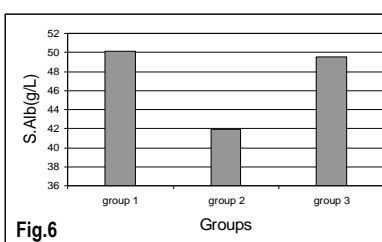


Figure.6: Shows serum Albumin levels in experimental groups.

Discussion

Intravenous injection of alloxan rapidly damages the β cells of the islets of langerhans in pancreas (Farjou and Lami, 1988; Koman *et al.*, 1991). If the pancreatic arterial supply is clamped for five minutes after the injection, the islets are protected (Akhtar *et al.*, 1984). The immediate effect of alloxan is the elevation of the blood glucose (Koman *et al.*, 1991). This elevation can be prevented by simultaneous treatment with insulin (Akhtar *et al.*, 1984). Prevention of the hyperglycemic phase does not prevent damage to the β cells, however, shortly after the initial hyperglycemic episode, there is a rapid drop of blood glucose to hypoglycemic levels, as a result of insulin released by the damaged β cells. Over the next few days, the blood glucose arises again and is usually maintained thereafter at elevated levels (Asyama *et al.*, 1989). At this point, β cells degenerated, and the insulin content of the pancreas is reduced to very low levels.

Destruction of pancreatic beta-cells by alloxan may results from reaction with glutathione or other sulfhydryl groups of proteins which would inactivate essential enzymes or coenzymes of the cell, alloxan injection may also results in generation of free radicals which cause breaking of DNA strands of beta-cells. Alloxan has also been shown to inactivate Ca^{+2} and calmodulin-dependent protein kinase, the activity of this enzyme was related to insulin secretion (Koman *et al.*, 1991).

The fall in concentrations of serum glucose in group (3) which was reported in the present study agreement with the study of (AKhani *et al.*, 2004) who found that treatment of streptozotocin – induced diabetic rats with ginger extract caused a significant decrease in the blood glucose and increased the insulin level (Kar *et al.*, 1999) reported that, the inorganic part of a medicinal plant contains mainly mineral elements, which are responsible for the hypoglycemic activity. In support of this view, a number of essential minerals (Ca, Zn, K, Mn and Cr) are known to be associated with the mechanisms of insulin release and its activity in different animals and in human beings (Castro, 1998).

The increase of the malondialdehyde concentration in group (2) as reported in the present study, this agree with the results of (Coli *et al.*, 2005). This rising in MDA level is directly associated with the degree of lipid peroxidation which is one of the most important measurement of oxidative stress in diabetes (Djeridane *et al.*, 2006).

The decrease of the malondialdehyde concentration in group (3) may be due to the main pharmacological actions of ginger and compounds isolated there from include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Ajay *et al.*, 2007).

The increase in concentration of serum iron in group (2) which were reported in the present study agree with the finding of (Wood *et al.*, 2004) and who showed that the toxic free radical types are superoxide radical anion (O^{-2}), the presence of the latter in high amount leads to releasing of free iron to circulatory system because O^{-2} attack to ferritin. While Lee *et al.* (2009) showed that the load of iron in patients with diabetes, and these findings was attributed to high oxidative stress in these patients to iron-derived free-radicals and to the patients diminished antioxidant reserve. An evidence suggests non-transferrin bound iron (NTBI) may be found in persons with diabetes (Hutchinson *et al.*, 2004). This (NTBI) has been associated with oxidative stress and chronic disease (Lee *et al.*, 2009).

The decrease in concentration of serum iron in group (3) which were reported in this study agree with the finding of (Golalipour *et al.*, 2007) that reported the decrease of iron levels in patients with diabetes as a result of treatment by phenolic extract (as anti-oxidants) can cause deficiency of oxidation processes, disruption of heme biosynthesis and low oxygen transfer might be resulted in a compensatory increase in the rate of red blood cell (RBC) production (Golalipour *et al.*, 2007). On the other hand (Thephiniap *et al.*, 2007) showed that phenolic compounds work by scavenging free radicals and quenching the lipid peroxidative chain. The hydroxy and phenoxy groups of phenolic compounds donate their electron to the free radicals and quench them. Besides, *in vitro* study of Thephiniap *et al.* (2007) had documented a protective effect of polyphenols like flavonoids on iron-induced oxidative stress. The decrease in concentrations of serum transferrin in group (2) which were reported in the present study agree with study of

Thabrew (2001) that concluded 86% of patients with diabetes develop hypoferremia. Besides, during diabetes, stimulation of phagocytes and activation of other immune complexes lead to further superoxide radical's production (Van Campenhout *et al.*, 2003). The latter attacks ferritin (iron storage protein) leads to release iron by reductive process.

The increase in concentration of serum transferrin in group(3) this may be due to the decrease of serum iron levels after treatment, our results is compatible with the previous study of (Biessels *et al.*, 2004) which showed that the decrease in serum iron levels associated with the increase of the binding capacity of transferrin to iron. Knekt *et al.*, (2002), suggested that polyphenols have combined effect on free iron that caused a decreased iron level. The increase in concentration of serum ceruloplasmin in group (2) was probably due to an increase in the proportion of younger red blood cells and the compensatory mechanism after increased oxidant stress (Rowe *et al.*, 1984). Besides, it was found to be as a result of the increase in catalysis of the liver cells synthesis of Cp against iron overload status (Tobe *et al.*, 2002) and elevation in serum copper level (Butric and Ashood, 1996) as a defiance function.

The decrease in concentration of serum ceruloplasmin in group (3), this may be due that plant polyphenols decrease an activity of numerous proteins associated with oxidative stress. Also, the reduction in Cp concentration could be to counter balance of the ROS generation radicals generated in the lipid peroxidation processes and presence of iron or copper ions (Sirjwala *et al.*, 2007). In the present work, we focused on the antioxidant activity of albumin because oxidative stress is thought to play a significant role in the pathogenesis of many diseases, including diabetes. Wolff *et al.* (2001) reported that the concentration of serum albumin decreased in diabetes because albumin is a carrier protein of copper and diabetic patients exhibit elevated concentration of copper ions that have been shown to generate free radicals. These highly reactive species are able to induce oxidative degradation of protein (Pacifici and Davies, 2001). Vlassara *et al.* (2001) reported that these decreasing are due to the increasing in synthesis of lipid peroxide and elevation formation of free radicals which result in increasing of membranes permeability and leaking the proteins outside the vascular system.

The results after treatment with plant oils pellet appear the increase in albumin levels, which is in agreement with the result of (Al-Hashem, 2009) who reported that polyphenols could induce decrease in lipid peroxidation processes as well as increase in the activities of plasma protein thiols as albumin and other serum proteins in both animal and human.

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