Hypoglycemic, antihyperlipidemic and antioxidant effects of ginger and alpha-lipoic acid in experimentally diabetic rats

Hend M. Tag[1*], AL Shimaa A. Ali [1], Moshira M.Abdelwahab [2], and Zohour I. Nabil[1]
[1] Zoology Department, Faculty of Sciences, Suez Canal University
[2] Biochemistry Department, Faculty of Medicine, Suez Canal University

ABSTRACT

Ginger and alpha-lipoic acid have recently gained attention as a potent antioxidant. The purpose of the present study was to investigate the possible therapeutic effects of daily oral administration of ginger and alpha-lipoic acid on diabetic rats. 42 Male albino rats (150-180gm) were divided into 7 groups; control, olive oil (vehicle) control, alloxan induced diabetic (DM), ginger-treated (GN), alpha-lipoic acid -treated (ALA), diabetic rats treated with ginger (DM+GN) and diabetic rats treated with alpha-lipoic acid (DM+ALA). After 5 weeks of GN and ALA administration (50 and 30 mg/kg b. wt respectively), hematological, biochemical and histological parameters were investigated. Diabetic rats exhibited a significant decrease in erythrocytic parameters as well as significant increase of fasting blood sugar (FBS), glycated hemoglobin (HbA1c), total cholesterol (TCh), triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and a decrease in the level of high density lipoprotein (HDL). In addition, diabetic rats showed a significant decrease in hepatic GSH and an increase in the content of malondialdehyde (MDA). The administration of GN and ALA caused a significant decrease in FBS, HbA1c, TCh, TG, and LDL- cholesterol, concomitant with a significant increase in HDL-cholesterol was recorded. Moreover, GN and ALA administration blunted the increase in MDA and stimulated the GSH production in the liver of diabetic rats. The findings of this study indicated that injection of GN and ALA corrected the erythrocytic parameters, lipid profile, blood sugar level in diabetic rats and showed antioxidative properties. Additionally, the therapeutic effect of GN and ALA was confirmed with histopathological examination of pancreas. It could be postulated that; (i) GN and ALA have protective and anti-hyperglycemic effects and (ii) overall anti-diabetic effects of ALA are better than those of GN, at least in this experimental condition.

Keyword: Ginger, Alpha-lipoic acid, Hypoglycemic, Lipid profile, antioxidants, alloxan, rats.

INTRODUCTION

Diabetes mellitus (DM) is a serious health problem which is responsible for many complications affecting various organs in the body and it is considered the third greatest cause of death all over the world (El-Hilaly et al., 2007). During the last twenty years, the prevalence of diabetes has increased dramatically in many parts of the world and the disease is now a worldwide public health problem (Pan et al., 2012). Despite remarkable progress in the management of diabetes mellitus by synthetic drugs, there has been a renewed interest in natural anti-diabetic agents, especially the medicinal plants that attributed with therapeutic virtues (Grover et al., 2002). High prevalence and long-term complications of diabetes mellitus have prompted a search for new oral hypoglycemic agents from such antidiabetic plants (Grover et al., 2002). Many plant extracts and plant products have been shown to have significant antioxidant activity as well as having hypoglycemic properties (Patel et al., 2012). Ginger (GN) is an underground rhizome of plant *Zingiber officinale* belonging to the family Zingibeaceae. It is considered now a common constituent of diet worldwide (Elshater et al., 2009). It was reported that ginger has medicinal properties against digestive disorders, rheumatism, and diabetes (Afzal et al., 2001). In addition, phytochemical reports have shown that the main constituents of ginger are Gingerol, Shagaols, Zingerone and Paradol (Li et al. 2012). Gingerol related compounds from ginger has been identified as an antioxidant (Masuda et al., 2004) Alpha-Lipoic acid (ALA) is a natural cofactor in dehydr ogenase complexes such as pyruvate dehydrogenase and

α-ketoglutarate dehydrogenase. It is a natural compound which widely present in plants and animals with antioxidant properties (Ghibu et al., 2009). ALA improves insulin sensitivity and is found to be useful in the treatment of insulin resistance (Evans and Goldfine, 2000). The therapeutic actions of LA are based on unique antioxidant ALA/ dihydrolipoic acid (DHLA) system. This system is able to reduce not only reactive oxygen species (ROS) but also has the ability to reduce and regenerate many crucial antioxidants including vitamin C, vitamin E and glutathione for this reason it is called as an antioxidant of antioxidants (Bilska et al., 2008). In the present study, the hypoglycemic, hypolipidemic and antioxidat properties of ginger and ALA were assessed in an animal model of diabetes.

MATERIALS AND METHODS

Chemicals

Alloxan monohydrate was obtained from sigma chemicals company, Egypt. Ginger (GN) tablets were obtained from the Arab Company for Pharmaceuticals and Medicinal plants (MEPACO), Egypt. Alpha lipoic acid powder was obtained from EVA Medical Company, Egypt. Thiobarbituric acid aqueous solution (TBA), n-butanol, pyridine, 1,1,3,3-tetramethoxypropane standard, trichloroacetic acid (TCA), phosphate buffer, 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), and reduced glutathione (GSH) standard were obtained from Fluka (Taufkirchen, Germany). All the chemicals used were analytical grade. The assay kits for cholesterol, triacylglycerol, low density lipoprotein

_____
* *Corresponding author:* hendmaarof1@yahoo.com

cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were obtained from BioDiagnostic Company, Giza, Egypt.

Animals and care

Forty two healthy adult male Sprague-Dawley rats (150 – 180 g) were obtained from the Animal Breeding House in Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. They were acclimatized to the environment for a week prior to the start of the experiments. The animals were kept in plastic cages under 12 h light/dark cycles with 25 ±3 °C. They were maintained on standard pellet diet and tap water *ad libitum.* The experiments were performed in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines (EC, 1986).

Induction of diabetes

Animals were fasted for 24 hours and injected with freshly prepared aqueous solution of alloxan monohydrate dissolved in saline (150 mg/kg, i.p.) as reported previously (Satyanarayana et al. 2001). After the injection, they had free access of food and water. Treated rats were allowed to drink 5% glucose solution after 6 hrs of alloxan injection to counter hypoglycemic shock. The diabetic state was assessed by measuring the non-fasting plasma glucose concentration 72 hrs after alloxan treatment using the AccuCheck device (Roche Diagnostics, Indianapolis, IN). Blood was collected from tail vein and rats with a blood glucose level above 250 mg/dl were selected for the study and considered as diabetics (Zhang et al., 2006).

Experimental Protocol

Animals were then randomly divided into seven groups consisting of 6 rats in each group; Group I (non-diabetic control receive saline); Group II (non-diabetic group receive olive oil; solvent of ALA); Group III (non-diabetic treated GN); Group IV(non-diabetic treated ALA); Group V (diabetic control); and Group VI (diabetic treated GN group); Group VII (diabetic treated ALA group). GN and ALA were given orally to the rats through a gastric tube daily for 5 weeks at a dose of 50 and 30 mg/kg b. wt, respectively.

Collection of blood and tissue samples.

At the end of the 5 weeks post-treatment, Animals were fasted overnight then Blood was withdrawn retro-orbitally from the inner canthus of the eye with the help of capillary tube under mild ether inhalation anesthesia (Fernández et al., 2010). Blood samples were collected in test tubes and allowed to clot for 10 min. Serum was separated by centrifuging the samples at 3000 rpm for 10 min and stored in a refrigerator until analyzed (at -20 ℃) until analyses of certain biochemical parameters. The animals were sacrificed after blood collection by cervical dislocation. The pancreas was then quickly

dissected out, washed in ice-cold saline and stored in 10% formalin for tissue characterization.

Hematological and biochemical analysis

Haematological parameters were evaluated by electronic haematological counter (selectra coulter, Germany). Blood glucose level was determined by using the AccuCheck device (Roche Diagnostics, Indianapolis, IN). Triglycerides (TG), total cholesterol (TCh), high-density lipoprotein cholesterol (HDL-Ch) and low-density lipoprotein cholesterol (LDL-Ch) were measured at the end of the treatment peroid. The concentrations of total cholesterol was determined using the method of Richmond (1973). HDL-cholesterol was measured by enzymatic colorimetric methods of Jacobs et al. (1990). Triglyceride was measured by the fossati method associated with trinder reaction (Fossati and Precipe, 1982), while very low density lipoprotein cholesterol (VLDL-Ch) was calculated as TG/5, and LDL-Ch was calculated using the formula of Friedewald et al. (1972), where LDL-Ch=TCh - [HDL-Ch + (TG/5)]. Liver tissues from different groups were minced and homogenized (10% w/v), separately, in icecold 1.15% KCl–0.01M sodium, potassium phosphate buffer (pH 7.4) with an electric homogenizer. The homogenate was centrifuged at 4,000g for 20min, and the resultant supernatant was used for oxidative stress parameters. The levels of reduced glutathione (GSH) were determined according to Ellman (1959). The levels of lipid peroxidation (LP) were determined by measuring the content of the thiobarbituric acid reactive substances (TBARS) in the tissue homogenates following the procedure of Hogberg et al. (1974). According to this method, TBARS assay values are usually reported in malonaldehyde (malondialdehyde, MDA) equivalents, a compound that results from the decomposition of polyunsaturated fatty acid lipid peroxides.

Histopathological examination

Pancreas samples were taken from rats in different treated groups, and then fixed in 10% formal saline solution for twenty four hours. Washing was done in tap water followed by serial dilutions of absolute ethyl alcohol for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in a hot air oven for twenty four hours. Paraffin wax tissue blocks were prepared for sectioning at 4 microns thickness by microtome. The obtained tissue sections were stained by hematoxylin and eosin stain for histopathological examination (Banchroft et al. 1996).

Statistical analysis

The results were presented as the mean ± standard error (SE) for six animals in each group. Statistically significant differences between groups were calculated using one- way analysis of variance (ANOVA) followed by post hoc multiple comparison test (Duncan). The

criterion for significance was set at p ≤ 0.05 (Field, 2000).

RESULTS

Normal levels of FBS and HbA1c in healthy adult rats were measured as 84.67±3.06 mg/dl and 3.00 ± 0.07, respectively. Alloxan treated rats showed a significant elevation in FBS and HbA1c levels with percent of change equal to 421.63 and 48.33 respectively as compared with control group. Administration of GN (50 mg/kg b.wt.) and ALA (30 mg/kg b.wt.) in alloxan treated rats caused significant reduction in blood glucose level (p≤ 0.05) as compared with diabetic control rats (Fig. 1, A). Also, they caused a significant reduction (p<0.05) in HbA1c as compared to diabetic rats (Fig. 1, B).
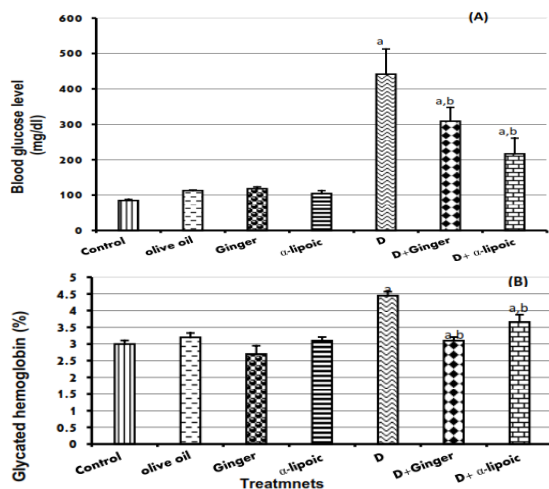

Figure (1): Effect of Ginger and αlipoic on (A) blood glucose and (B) glycated hemoglobin level in normal and alloxan induced diabetic rats. Values as mean ± SE. n=6, One Way ANOVA followed by Duncan multiple comparison tests. [a] p<0.05 when compared with normal control group, [b]p<0.05 when compared with diabetic (D) group.

As shown in Table 1, there was no significant change in erythrocytic parameters in normal rats treated with

either GN or ALA. In Alloxan-diabetic rats, there were a significant decrease in red blood cell count, Hb, PCV, MCV, MCH and MCHC. These parameters were however significantly reversed by administration of GN and ALA when compared to the untreated diabetic group. However, GN and ALA were not able to restore the level of MCH back to that obtained in control group.

Table 2 showed the results of lipid profile of plasma in control and diabetic rats treated with GN or ALA. The data revealed that lipid profile was impaired in alloxan-diabetic rats where the levels of TCh, TG, LDL, and VLDL were significantly increased while the level of HDL was significantly (P ≤ 0.05) reduced compared with normal control group. On the other hand, treatment of diabetic rats with ALA significantly increased the level of HDL by 30%, while LDL was significantly (P ≤ 0.05) reduced by 61.51%. In addition, ALA treatment significantly decreased the level of TCh, TG, and VLDL levels in diabetic rats when compared with diabetic control rats. The oral administration of GN to diabetic rats resulted in significant declines (P < 0.05) in plasma total cholesterol (- 21.96%) and LDL-Ch (- 43.25%) while, there was an increase (29.57%) in HDL-Ch in comparison to diabetic rats. The TG and VLDL of diabetic rats treated with GN showed no significant difference compared with diabetic control rats. However, antihyperlipidemic effects of ALA are better than those of GN.

A significant increase in liver MDA levels were observed in diabetic animals compared with non-diabetic animals (Fig 2). Meanwhile, the level of GSH was decreased significantly in the diabetic control group compared to the normal control group. On the other hand, significant increase in GSH level associated with significant decrease of MDA values; were evident in diabetic group treated with GN and ALA compared with diabetic untreated animals. Antioxidative effects ALA are better than those of GN (Fig 2). The light microscopic examination of pancreas in control rats showed normal histological structure of the island of Langerhans cells as endocrine cells and the acini as exc-

Table (1): Effect of Ginger and α-lipoic on erythrogram in alloxan diabetic rats

Groups	Red blood cell count (10^6/ μL)	PCV %	Hb (g/dL)	MCV (fL)	MCH (Pg)	MCHC %
Control	8.55± 0.30	46.76 ± 2.22	14.13 ± 0.62	54.60 ± 1.90	16.50 ± 0.18	31.35 ± 0.35
Olive oil	9.10± 0.07	45.20 ± 0.18	14.03 ± 0.09	55.66 ± 0.52	16.43 ± .021	31.03 ± 0.12
GN	7.90 ± 0.14 [a]	44.30 ± 0.47	13.75 ± 0.41[a]	56.10 ± 0.63[b]	16.30 ± 0.41	31.6 ± 0.21[a]
ALA	8.28 ± 0.33	43.50 ± 1.03	13.26 ± 0.43	54.86 ± 1.99	16.08 ± 0.32	31.50 ± 0.73
D	7.15 ± 0.24 [a]	40.50 ± 0.35 [a]	11.10 ± 0.32 [a]	49.60 ± 1.80 [a]	15.50 ± 0.19 [a]	27.40 ± 0.88 [a]
D +GN	8.61 ± 0.30 [b]	43.87 ± 1.69 [b]	13.53 ± 0.53[b]	55.95 ± 0.86 [b]	15.97 ± 0.26	31.35 ± 0.37[b]
D + ALA	8.45± 0.15 [b]	42.30 ± 1.26 [b]	13.00 ± 0.36[b]	53.96 ± 0.70 [b]	16.00 ± 0.35	30.06 ± 0.41 [b]

Values as mean ± SE. n=6, One Way ANOVA followed by Duncan multiple comparison tests. [a] p<0.05 when compared with normal control group, [b]p<0.05 when compared with diabetic (D) group.

Table (2): Effect of Ginger and α-lipoic on lipid profile in alloxan diabetic rats

Groups	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)
Control	67.63 ± 2.72	37.70 ±1.42	13.33 ± 0.42	49.80 ± 3.69	9.96± 0.73
Olive oil	61.30 ± 3.38	31.67± 1.29	10.67 ± 0.76	66.20 ± 9.67	13.24± 1.93
GN	76.95 ± 6.35	37.97 ± 2.60	13.17 ± 1.68	61.70 ± 7.20	12.34± 1.44
ALA	71.28 ± 8.03	36.40 ± 2.30	15.00 ± 2.63	46.31 ± 10.07	9.26± 2.01
D	104.00 ± 0.36 [a]	30.00 ± 3.01 [a]	24.67 ± 1.47 [a]	117.00 ± 0.36 [a]	23.40± 0.07 [a]
D +GN	81.16 ± 10.42 [b]	38.87 ± 4.28 [b]	14.00 ± 1.69 [b]	99.12 ± 23.90 [a]	19.82± 4.27 [a]
D + ALA	61.68 ± 6.50 [b]	39.00 ± 3.01 [b]	9.50 ± 0.96 [b]	54.53 ± 5.40 [b]	10.91± 1.08 [b]

Values as mean ± SE. n=6, One Way ANOVA followed by Duncan multiple comparison tests. [a] $p<0.05$ when compared with normal control group, [b]$p <0.05$ when compared with diabetic (D) group.

ocrine (Figure 3A). Pancreatic tissue from GN and alpha-lipoic treated non diabetic rats displayed normal structure (Figure 3C and 3E, respectively). The alloxan diabetic rats showed atrophy in the island of Langerhans cells (Figure 3B). However, treatment of alloxan-diabetic rats with either GN or ALA displayed recovery in size of island of Langerhans (Figure3 D and F, respectively).
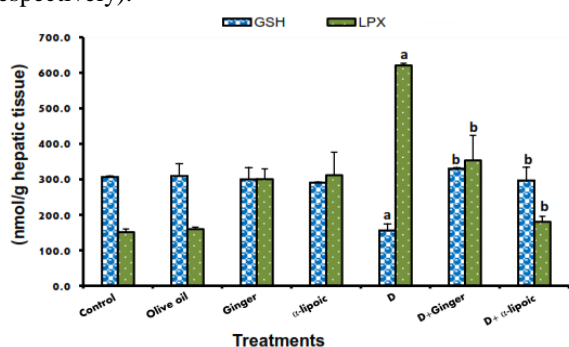

Figure (2): Effect of Ginger and αlipoic on liver glutathione and lipid peroxidation content in normal and alloxan induced diabetic rats. Values as mean ± SE. n=6, One Way ANOVA followed by Duncan multiple comparison tests. [a] $p<0.05$ when compared with normal control group, [b]$p<0.05$ when compared with diabetic (D) group.
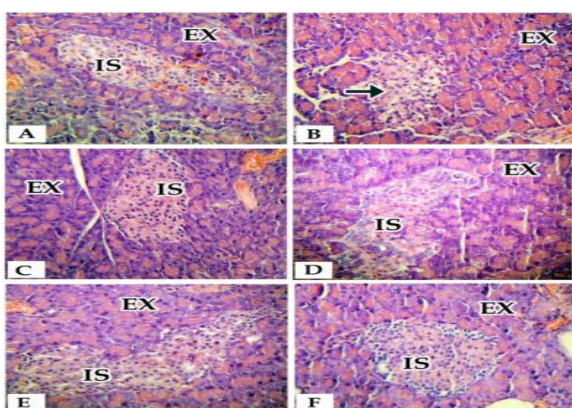

Fig 3: Histopathological examination of pancreas section: (A) control rats (B) diabetic rats displayed atrophied islets (arrow) (C) GN treated rats (D) Diabetic rats treated with GN (E) ALA treated rats (D) Diabetic rats treated with ALA. (EX): exocrine gland; (IS): islets of Langerhans. (H&E; 200X).

DISCUSSION

In the present study alloxan diabetic rats receiving GN or ALA showed a significant reduction of their fasting blood glucose values in comparison to diabetic control rats. The results are in consistent with Sharma and Shukla, (1977) who reported a significant blood glucose lowering effect of ginger juice in diabetic and non-diabetic animals. The results are also in corroboration with Sharma et al. (1996); Ahmed and Sharma, (1997) who reported hypoglycemic effects of ginger extract in diabetic induced animals. In addition, Akhani et al., (2004) stated that ginger pretreatment inhibited the induced hyperglycemia and hypoinsulinaemia. Recent phytochemical studies have shown that the hypoglycaemic effect of ginger is due to presence of tannins and polyphenols with anti-oxidant property (Ugwoke and Nzekwe, 2012).

Oral administration of ALA for a period of five weeks significantly reduced the FBG in the alloxan-diabetic rats. This gave an indication of possible effects on the blood glucose metabolic pathway. Most authors who have reported on glycemic control potential of ALA have linked it with its ability to recruit glucose transporter-4 to plasma membranes similar to the insulin-stimulated glucose uptake (Singh and Jialal, 2008; Morakinyo et al., 2013).

A number of studies had shown significantly increased glycosylated hemoglobin levels in diabetic rats than the normal controls as seen in this study (Moussa, 2008; Ojo et al., 2013). Glycosylation is the interaction of glucose with the N- terminal amino acid of hemoglobin, producing glycosylated hemoglobin. Normally about one third of hemoglobin is glycosylated while it rises to two or to three folds in diabetic patients. It persists all through the life span of RBC. Hence, estimation of glycosylated hemoglobin is an important diagnostic tool to assess the long-term control of blood glucose (WHO., 1999). H_2O_2 is produced as ROS and causes the release of iron from HbA1c more readily than normal hemoglobin. The iron is responsible for the generation of free radicals, which degrades various cell constituents. They also interact with DNA and proteins and bring out detrimental changes in their structure and properties. HbA1C is also subjected to auto-oxidation

generating further oxidative stress (Aparna et al., 2012). In the present study, administration of GN effectively reduced HbAlc levels in diabetic rats. These results are in corroboration with Khadem Ansari et al. (2008) who showed decreased HbAlc levels by administration of ginger extract to the diabetic animals and this may be due to the decreased level of blood glucose.

The present results in agreement with a number of studies who reported that the enhancement of haematological parameters like MCH, MCV PCV, red blood cell count and Hb after GN (Ademola et al., 2009; Haghighi and Rohani, 2013) and ALA treatment (Bouasla et al., 2014) showed that GN and ALA can be useful in the treatment of anaemia and other blood disorders. The interpretation of hematological parameters enhancement after treatment with natural remedies has been subjected to wide speculation by many investigators. Tahmasebpour et al., (2013) reported that the increase in RBC, PCV, and WBC counts following administration of the methanol extract of *T. orientale* may signify the positive effects on the haemopoietic system of experimental rats and might be capable of improving the hematological abnormalities associated with pathophysiology of diabetes mellitus. Similarly, it can be suggested that GN and ALA has a stimulatory effect on the bone marrow, which is responsible for production of red blood cells.

The present results elucidated that the rise in blood glucose was accompanied with marked increase in total lipids, triglycerides, total cholesterol and LDL-Ch in diabetic rats. These data were confirmed with the results of Battell *et al.* (1998) and Abdel-Moneim *et al.* (2002) who reported that, marked elevation in serum triglycerides, cholesterol and LDL-cholesterol levels in diabetic animals. This due to decrease in lipoprotein lipase (LPL) activity resulted from insulin deficiency (Minnich and Zilversmit, 1989). Diabetic dislipidemia is due mainly to decreased removal of triglycerides into the fat depots and the increase in the plasma concentration of LDL-cholesterol (Tsutsumi *et al.*, 1995). According to the present results, there was a significant decrease in the levels of triglycerides and cholesterol after the administration of GN and ALA in comparison with diabetic animals. Hypolipidaemic effect of ginger extract was also demonstrated in cholesterol-fed rabbits (Bhandari and Grover, 1998). This may be an indication of progressive metabolic control of ginger on mechanisms involved in elimination of the lipids from the body. This hypolipidemic properties have been confirmed in many plant species and plant products in medicinal use (Kono et al., 1992; Naidu and Thippeswamy, 2002; Devi and Sharma, 2004). A variety of phenolic compounds, in addition to flavonoids, are found in ginger. It was concluded that the hypocholesterolaemic effect of ginger could have possibly resulted from the inhibition of cellular cholesterol biosynthesis after the consumption of the ginger (Fuhrman et al., 2000). Akhani et al., (2004) reported that ginger treatment

significantly decreased both serum cholesterol and triglycerides. In addition, Bhandari et al., (2005) found that, the ethanolic extract of ginger significantly reduced serum total cholesterol and triglycerides and increased the HDL-Ch levels. Also, the extract can protect tissues from lipid peroxidation and exhibit a significant lipid lowering activity in diabetic rats. In an attempt for interpretation this phenomena, Matsuda et al. (2009) revealed that ginger modifies lipid metabolism by inhibiting cellular cholesterol biosynthesis, increasing bile acid biosynthesis to eliminate cholesterol from the body and increasing fecal cholesterol excretion. Ginger has been shown to reduce the serum levels of cholesterol and triglycerides in fructose-induced hyperlipidemic rats (Kadnur and Goyal, 2005). Moreover, Alizadeh et al. (2008) indicated that, ginger has a significant lipid lowering effects in patients with hyperlipidemia. In contrast, in another study, non-diabetic patients with coronary artery disease showed no decrease in their blood lipid or sugar levels when treated with a daily dose of 4 g powdered ginger for a period of 3 months (Bordia et al., 1997).

Hyperglycemia is widely known to enhance free radical concentrations and decreased antioxidant defense system (Matough et al., 2012). This mechanism may occur via at least four different routes: increased glycolysis; intercellular activation of sorbitol (polyol) pathway; auto-oxidation of glucose and non-enzymatic protein glycation (Ahmed 2005). Previous studies have shown that the increase in oxidative stress in diabetes is due to the increased production of ROS and decreased antioxidant defense system (Wiernsperger, 2003). Studies by Annuziata et al. (2005) showed that tissue injury due to free radical damage acts as an important factor in the pathogenesis and complication of diabetes mellitus. The stability and capacity of the antioxidant status during chronic diabetes seriously influences the outcome of the long-term complications caused by oxidative stress (Sasvari and Nyakas, 2003).

In the present study the observed significant increase in liver MDA and a decrease of GSH content in diabetic rats could be due to destruction of pancreatic ß cells by alloxan probably through the generation of oxygen free radicals (Yang et al., 2010). Ginger decreases MDA concentration in normal, alloxan-induced and insulin resistant diabetic rats (Ajith et al. 2008; Heeba and Abd-Elghany, 2010; Oboh et al., 2010), as it acts as a scavenger of oxygen radicals and also acts as an antioxidant.

Ginger extract possesses antioxidative characteristic since it can scavenger superoxide anion and hydroxyl radicals (Krishnakantha and Lokesh, 1993). Malondialdehyde (MDA) is the end-product of lipid peroxidation and the production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism (Oboh, et al. 2010). Increased oxidative stress and decreased antioxidant levels are the leading cause of diabetes and diabetic complications (Jain, et al.

1996; Feillet-Coudray, et al. 1999). Literature has shown that *Zingiber officinale* exhibit anti-oxidant effects (Stoilova et al. 2007; Ghasemzadeh, et al. 2010; Heeba & Abd-Elghany, 2010) which can be classified as a source of natural or phytochemical antioxidants (Kikuzaki and Nakatani, 1993). Treatment with 30 mg/kg α-lipoic acid for 5 weeks prevented the increase of MDA and increase hepatic GSH in diabetic rats. Feng et al. (2013) verified that one of the mechanisms by which ALA exerts this protective effect is via decreasing oxidative stress.

The present work concluded that the effect of GN and ALA administration on alloxan-induced diabetic rat showed hypoglycemic and antioxidant effects by enhancing liver GSH and down regulating the malondialdehyde (MDA) level. Also GN and ALA reduced significantly the plasma total cholesterol with its LDL- fraction. Therefore, it may be regarded as a useful therapy for hyperlipidemia and the lowering of circulating cholesterol and the increased in HDL cholesterol

REFRENCES

ABDEL-MONEIM, A., E. AL-ZAYAT AND S. MAHMOUD. 2002. Effect of some antio-xidents on streptozotosin diabetic rats comparative physiology Egyptian German Society Of Zoology, **38**(A):213-245.

ADEMOLA S.G., G.O. FARINU AND G.M. BABATUNDE. 2009. Serum Lipid, Growth and Haematological Parameters of Broilers Fed Garlic, Ginger and Their Mixtures. World Journal of Agricultural Sciences. **5**(1): 99-104.

AFZAL, M., D.AL HADIDI, M., MENON, J. PESEK, AND M.S. DHAMI. 2001. Ginger: an ethnomedical, chemical and pharmacological review. Drug Metabolism and Drug Interactions **18:**159-190.

AHMED, R. G. 2005. The physiological and biochemical effects of diabetes on the balance between oxidative stress and antioxidant defense system. Medical Journal of the Islamic World Academy of Sciences. **15:** 31-42.

AHMED, R.S. AND S.B. SHARMA. 1997. Biological studies on combined effects of garlic (Allium sativum Linn) and ginger (Zingiber officinale Roscoe) in albino rats. Journal of Experimental Biology. **35:**841-843.

AJITH, T. A., M. S. ASWATHY AND U. HEMA 2008. Protective effect of Zingiber officinale roscoe against anticancer drug doxorubicin-induced acute nephrotoxicity. Food and Chemical Toxicology. **46**(9). 3178 – 3181.

AKHANI, S. P., S.L. VISHWAKARMA, AND R. K. GOYAL. 2004. Anti-diabetic activity of Zingiber officinale in Streptozotocin-induced type I diabetic rats. Journal of Pharmacy and Pharmacology **56:** 101-105.

ALIZADEH, R.F., F. ROOZBEH, M. SARAVI, M. POURAMIR, F. JALALI, A.A. MOGHADAMNIA 2008. Investigation of the effect of ginger on the lipid levels. A double blind controlled clinical trial. Saudi Journal of Medicine., **29**(9): 1280-1284.

ANNUZIATA, L., F. DOMENICO AND T. PIETRO 2005. Glyco-oxidation in diabetes and related disease. Clin Chim Acta, **2:** 236-250.

APARNA. R. R, B. SUNEEL, B. SOWJANYA AND A. D. BALAKRISHN. 2012. Study on orrelation between glycated heamoglobin and oxidative stress in diabetics. International Journal of Pharma and Bio Sciences. **3**(2): B – 337- B - 343

BANCHROFT, J., A. STEVENS AND D. TURNER. 1996. Theory and practice of histological techniques. 4th ed. Churchil Livingstone, New York, London, San Francisco, Tokyo

BATTELL M. L., DELGATTY H. L. M. AND J. H. MCNRILL (1998): Sodium selenate corrects glucose tolerance and heart function in STZ diabetic rats. Molecular and Cellular Biochem **179:**27-34.

BHANDARI, U., J. N. SHARMA, AND R. ZAFAR. (1998). The protective action of ethanolic ginger (Zingiber officinale) extract in cholesterol-fed rabbits. J.Ethnopharmacol **61:**167-171.

BHANDARI, U., R. KANOJIA AND K.K. PILLAI 2005. Effect of ethanolic extract of Zingiber officinale on dyslipidaemia in diabetic rats. J Ethnopharmacol **97:** 227 – 230.

BILSKA, A., M. DUDEK, M. ICIEK, I. KWIECIEN, M.SOKOLOWSKA-JEZEWICZ, B. FILIPEK AND L. WLODEK 2008. Biological actions of lipoic acid associated with sulfane sulfur metabolism. Pharmacological Reports **60:** 225- 232.

BORDIA, A., S. K. VERMA AND K. C. SRIVASTAVA. 1997. Effect of ginger (Zingiber officinale Rosc.) and fenugreek (Trigonella foenumgraecum L.) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease. Prostaglandins. Leukot. Essent. Fatty Acids, **56**(5): 379-384

BOUASLA I., A. BOUASLA, A. BOUMENDJE, A. EL FEKI, M. MESSARAH. 2014. Antioxidant Effect of Alpha Lipoic Acid on Hepatotoxicity Induced by Aluminium Chloride in Rats. International Journal of Pharmaceutical Sciences Review and Research **29**(2): 19-25

DEVI, R. AND SHARMA, D. K. 2004. Hypolipidemic effect of different extracts of Clerodendron colebrookianum Walp in normal and high-fat diet fed rats. J Ethnopharmacol **90:**63-68.

EL-HILALY, J., T. ADIL, H. I. ZAFAR AND L. A. BADIÂA 2007. Hypoglycemic, hypocholesterolemic and hypotriglyceridemic effects of continuous intravenous infusion of a lyophilized aqueous extract of ajuga iva l. Schreber whole plant in streptozotocin-induced diabetic rats. Pakistan journal of pharmaceutical sciences **20**(4): 261-268.

ELLMAN, G. L. 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics., **82:**70-77.

ELSHATER, A. E., M. A. SALMAN, M. A. MOUSSA 2009. Effect of Ginger Extract Consumption on levels of blood Glucose, Lipid Profile and Kidney Functions in Alloxan Induced-Diabetic Rats. Egyptian Academic Journal of Biological Sciences., **2**(1): 153-162

EUROPEAN COMMUNITIES (EC) 1986. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Off. J. Eur. Communities, L 358, 18/12/1986, 1-28

EVANS, J. L., AND I. D. GOLDFINE. 2000. α-Lipoic acid a multifunctional antioxidant that improves insulin sensitivity in patients with type 2 diabetes. Diabetes Technology & Therapeutics **2**: 401–413.

FEILLET-COUDRAY, C., E. ROCK, C. COUDRAY, K. GRZELKOWSKA, V. AZAIS-BRAESCO, D. DARDEVET, A. MAZUR. 1999. Lipid peroxidation and antioxidant status in experimental diabetes. Clinica Chimica Acta. **284**(1): 31 – 43.

FENG, B., X. YAN, J. XUE, L. XU AND H. WANG. 2013. The Protective Effects of α-Lipoic Acid on Kidneys in Type 2 Diabetic Goto-Kakisaki Rats via Reducing Oxidative Stress. International Journal of Molecular Sciences 14:6746-6756.

FERNÁNDEZ, I., A. PEÑA, N. DEL-TESO, V. PÉREZ AND J. RODRÍGUEZ-CUESTA. 2010. Clinical Biochemistry Parameters in C57BL/6J Mice after Blood Collection from the Submandibular Vein and Retroorbital Plexus. Journal of the American Association for Laboratory Animal Science, **49**(2): 202-206

FIELD, A.P. 2000. Discovering statistics using SPSS for windows: advanced techniques for the beginner. Sage publications, London.

FOSSATI, P., L. PRECIPE. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry **28:** 2007-2080.

FRIEDEWALD, W.I., R. I. LEVY AND D.S. FREDRICKSON 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry., **18**(6): 499-502.

FUHRMAN, B., M. ROSEBLATE, T. HAYEK, R. COLEMAN AND M. AVIRAM 2000. Ginger Extract Consumption Reduces Plasma Cholesterol, Inhibits LDL Oxidation and Attenuates Development of Atherosclerosis in Atherosclerotic, Apolipoprotein E-Deficient Mice. Journal of Nutrition. **130:** 1124-1131.

GHASEMZADEH, A., H. Z. E. JAAFAR AND A. RAHMAT. 2010. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysian young ginger (Zingiber officinale Roscoe). Molecules **15:** 4324 – 4333.

GHIBU S., C. RICHARD, C. VERGELY, ZELLER M., COTTIN Y., ROCHETTE L. 2009. Antioxidant properties of an endogenous thiol: Alpha-lipoic acid,

useful in the prevention of cardiovascular diseases. J Cardiovascular Pharmacology **54**(5):391-8.

GROVER, J. K., S. YADAV AND V. VATS. 2002. Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol **81:** 81 – 100.

HAGHIGHI, M. AND M. S. ROHANI. 2013. The effects of powdered ginger (Zingiber officinale) on the haematological and immunological parameters of rainbow trout Oncorhynchus mykiss. Journal of Medicinal Plant and Herbal Therapy Research **1:** 8-12

HEEBA, G.H. AND M. I. ABD-ELGHANY 2010. Effect of combined administration of ginger (Zingiber officinale Roscoe) and atorvastatin on the liver of rats. Phytomedicine **17**(14):1076 – 1081.

HOGBERG, J., R. E. LARSON AND A. KRISTOFFERSON 1974. NADPH-dependent reductase solubilized from microsomes by peroxidation and its activity. Biochemical and Biophysical Research Communications **56:** 836-842.

JACOBS, D.S, B. L. KASTEN, W.R. DE MOTT AND W.L. WOLFSON. 1990. In laboratory and Test Handbook; Eds ; Lexi-comp Inc: Hudson, Cleveland; p. 219.

JAIN, S.K., R. MCVIE, J.J. JARAMILLO, M. PALMER, T. SMITH, Z. D. MEACHUM AND R. L. LITTLE. 1996. The effect of modest vitamin E supplementation on lipid peroxidation products and other cardiovascular risk factors in diabetic patients. Lipids. Suppl. S 87 – 90.

KADNUR S.V. AND R. K. GOYAL. 2005. Beneficial effects of Zingiber officinale Roscoe on fructose induced hyperlipidemia and hyperinsulinemia in rats. Indian journal of experimental biology, **43**(12): 1161-1164.

KIKUZAKI, H. AND N. NAKATANI. 1993. Antioxidant effect of some ginger constituents. J. Food Sci. **578**: 1407 – 1410.

KONO, S., K. SHINCHI, N. IKEDA, F. YANAI AND K. IMANISHI. 1992. Green tea consumption and serum lipid profiles: a cross-sectional study in northern Kyushu, Japan. Preventive Medicine **21:** 526-531.

KRISHNAKANTHA, T. P. AND B. R. LOKESH. 1993. Scavenging of superoxide anions by spice principles. Indian Journal of Biochemistry and Biophysics 30: 133-134.

LI, Y., V. H. TRAN, C. C. DUKE, AND B. D. ROUFOGALIS. 2012. Preventive and Protective Properties of Zingiber officinale (Ginger) in Diabetes Mellitus, Diabetic Complications, and Associated Lipid and Other Metabolic Disorders: A Brief Review Evidence-Based Complementary and Alternative Medicine Volume 2012, Article ID 516870, 10 pages doi:10.1155/2012/516870

MASUDA Y., KIKUZAKI H., HISAMOTO M., NAKATANI N. 2004. Antioxidant properties of gingerol related compounds from ginger. Biofactors **21**(1-4):293-6.

MATOUGH FA., S.B. BUDIN, Z.A HAMID, N. ALWAHAIBI, AND J. MOHAMED 2012. The Role of Oxidative Stress and Antioxidants in Diabetic Complications. Sultan Qaboos University Medical Journal **12**(1): 5–18.

MATSUDA, A., Z. H. WANG, S.H. TAKAHASHI, T.

TOKUDA, N. MIURA AND J. HASEGAWA. 2009. Up regulation of mRNA of retinoid binding protein and fatty acid binding protein by cholesterol enriched-diet and effect of ginger on lipid metabolism. Life Sciences **84**(25-26): 903-907.

MINNICH, A. AND D. B. ZILVERSMIT. 1989. Impaired triacylglycerol catabolism in hypertriglyceridemia of the diabetic, chole-sterol fed rabbit: A possible mechanism for protection from atherosclerosis. Biochimica et Biophysica Acta **1002:** 324-332.

MORAKINYO, A. O., F. O AWOBAJO, O. A ADEGOKE. 2013. Effects of alpha lipoic acid on blood lipids, renal indices, antioxidant enzymes, insulin and glucose level in streptozotocin-diabetic rats Biology and Medicine **5**: 26–33, 2013

MORAKINYO, A.O., O. S. ADENIYI AND A. P. ARIKAWE. 2008. Effects of Zingiber officinale on reproduvtive functions in male rats. African Journal of Biochemistry Research **11**: 329 – 333.

MOUSSA, S.A. 2008. Oxidative stress in diabetes mellitus. Romanian Journal of Biophysics **18**: 225-236.

NAIDU, K.A., N. B. THIPPESWAMY. 2002. Inhibition of human low density lipoprotein oxidation by active principles from spices. Molecular and Cellular Biochemistry **229**(1–2):19–23.

OBOH, G., A. J. AKINYEMI AND A. O. ADEMILUYI. 2010. Antioxidant and inhibitory effect of red ginger (Zingiber officinale var. Rubra) and white ginger (Zingiber officinale Roscoe) on Fe (2+) induced lipid peroxidation in rat brain in vitro. Experimental and Toxicologic Pathology. 2010 (1).

OJO, R. J., P. I. OGUCHE, G. D. KUBE AND T. E. UDZER. 2013. Effect of Jatropha curcas supplemented diet on broilers. Scholars Acad. J. Biosci **1**(6):329-336

PATEL, D.K., S. K. PRASAD, R. KUMAR AND S. HEMALATHA 2012. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pacific Journal of Tropical Biomedicine. **2**(4): 320-330

RICHMOND, W. 1973. Preparation and properties of a cholesterol oxidase from nocardia sp. And its application to the enzymatic assay of total cholesterol in serum.Clin.Chem. **12**: 1350-1356.

SASVARI, M. AND C. NYAKAS. 2003. Time dependent changes in oxidative metabolism during

chronic diabetes in rats. Acta. Biologica. Szegediensis **47:** 153-158.

SATYANARAYANA, K., V. MANGATHAYARU, J. SREEKANTH, V. VENKATESWARLU AND C. K. KOKATE. 2001. Studies on hypoglycemic and cardiotonic effects of roots of Cocculus hirsatus. Indian Journal of Pharmaceutical Sciences **63:** 30-35

SHARMA, I., D. GUSAIN AND V. P. DIXIT 1996. Hypolipidemic and antiatherosclerotic effects of Zingiber officinale in cholesterol-fed rabbits. Phto. Res. **10:**517-518.

SHARMA, M. AND S. SHUKLA. 1977. Hypoglycaemic effect of ginger. The J. of Research in Indian Yoga and Homoeopathy **12:** 127–130.

SINGH, U. AND I. JIALAL. 2008. Alpha lipoic acid supplementation and diabetes. Nutrition Review **66**(11): 646–657.

STOILOVA, I., A. KRASTANOV, A. STOYANOVA. 2007. Antioxidant activity of a ginger extract (Zingiber officinale). Food Chemistry **102**. 764 – 770.

TAHMASEBPOUR N., G. DEHGHAN, M.A.H. FEIZI AND H.A. ESMAEILI, 2013.Variation in body weight and some hematological parameters in streptozotocin-induced diabetic rats, treated with Teucrium orientale . harmacologyOnLine.**3**:32-36

TSUTSUMI K., Y. INOUE, A. SHIME AND T. MURASE. I995. Correction of hypertriglyceridezna with low and high-density lipoprotein cholesterol by the novel compound No. 1886, a lipoprotein lipase-promoting agent, in STZ-induced diabetic rats. Diabetes **44:**414-417.

UGWOKE, C. E. C. AND U. NZEKWE. 2010. Phytochemistry and proximate composition of ginger (Zingiber officinale). Journal of Pharmaceutical and Allied Sciences, Vol. **7** No. 5 (2010)

WIERNSPERGER, N.F. 2003. Oxidative stress as a therapeutic target in diabetes: revisiting the controversy. Diabetes & Metabolism **29:** 579–585.

WORLD HEALTH ORGANIZATION. 1999. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus. WHO/NCD/NCS/99.2 ed. Geneva, World Health Organization.

YANG, C.Y., J. WANG, Y. ZHAO, L. SHEN, X. JIANG, Z.G. XIE N. LIANG, L. ZHANG, Z.H CHEN. 2010. Anti-diabetic effects of Panax notoginseng saponins and its major anti-hyperglycemic components. J. Ethnopharmacol. 2010 Jul 20;**130**(2): 231 pp.

ZHANG, J., Y. HUANG, T. HOU AND Y. WANG. 2006. Hypoglycemic effect of Artemisia sphaerocephala Krasch seed polysaccharide in alloxan-induced diabetic rats. SWISS. MED. WKLY., **136:** 529-532.

.

التأثيرات المخففة لنسبة السكر والدهون ومضادات الأكسدة للزنجبيل وحمض الفا- ليبويك في الجرذان المصابة بداء بالسكري المستحث بالالوكزان

هند معروف تاج¹ ، الشيماء أحمد أحمد علي¹ ، مشيرة عبد الوهاب² ،زهورابراهيم نبيل¹

¹قسم علم الحيوان، كلية العلوم، جامعة قناة السويس

²قسم الكمياء الحيوية، كلية الطب البشري، جامعة قناة السويس

ظهر في الآونة الأخيرة الإهتمام بالزنجبيل و حمض ألفا- ليبويك ـ بإعتبارها أحد مضادات الأكسدة القوية. والغرض من هذا البحث هو دراسة آثار تناول الزنجبيل وحمض الفا- ليبويك عن طريق الفم يوميا على قياسات الكريات الحمراء ومستوى السكر في الدم والهيموجلوبين السكري و مستوي الدهون و الجلوتاثيون واكسدة الدهون في الكبد وكذلك التأثيرات النسيجية المرضية علي البنكرياس في الجرذان الغير مصابة والمصابة بالسكري. تم تقسيم عدد ٤٢ من ذكور الجرذان البيضاء (١٥٠-١٨٠gm) إلى ٧ مجموعات. (١) المجموعة الضابطة، و(٢) المجموعة المعالجة بزيت الزيتون، و(٣) المجموعة الألوكزان الذي يسبب السكري و المجموعة ٤ و٥ تم معالجتها بالزنجبيل، و حمض الفا- ليبويك .المجموعة ٦ و٧ تمثل الجرذان المصابة بداء السكري والتي تم معالجتها بالزنجبيل أو حامض ألفا- ليبويك. بعد ٥ أسابيع من المعالجة أظهرت الجرذان المصابة بداء السكري حدوث إنخفاض كبير في قياسات الكريات الحمراء، فضلاً عن زيادة كبيرة في مستوي السكر في الدم، الهيموجلوبين السكري، الكولسترول الكلي، الدهون الثلاثية، البروتين الدهني منخفض الكثافة، و البروتين الدهني ذو الكثافة المنخفضة جدا، وإنخفاض في مستوى البروتين الدهني عالي الكثافة. كما أظهرت الفئران المصابة بالسكري إنخفاض ملحوظ في مستوي الجلوتاثيون في الكبد وزيادة في محتوى أكسدة الدهون. أظهرت معالجة الجرذان المصابة بالسكري بكلاً من الزنجبيل وحامض الالفا- ليبويك إنخفاضا كبيرا في مستوي السكر في الدم ، الهيموجلوبين السكري، الكولسترول الكلي، الدهون الثلاثية، البروتين الدهني منخفض الكثافة، و البروتين الدهني ذو الكثافة المنخفضة جدا، و زيادة كبيرة في الكولسترول ذا الكثافة المرتفعة. أيضا، قلل الزنجبيل وحامض الالفا- ليبويك الزيادة في مستوي اكسدة الدهون بنسيج الكبد وحفز إنتاج الجلوتاثيون في الحيوانات المصابة بالسكري أظهرت القطاعات المجهرية لأنسجة البنكرياس تحسنٌ فى شكل وترتيب الخلايا بيتا والمسئولة عن إفراز الإنسولين . نتائج هذه الدراسة تشير إلى أن معالجة مرض السكري باستخدام الزنجبيل أو حامض الالفا ليبويك لها تأثيرات مخففة لزيادة نسبة الجلوكوز والدهون بالدم ولكن أوضحت هذه الدراسة أن فاعلية حامض الالفا- ليبويك أكثر من الزنجبيل في علاج مرض السكري.