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EVALUATION OF ANTIDIABETIC AND INSULINTROPIC POTENTIAL OF NIGELLA SATIVA SEEDS WATER EXTRACT AND /OR ALPHA LIPOIC ACID IN TYPE 2 DIABETIC RATS

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Keywords:

Nigella sativa;AlphaLipoic acid ;type 2 diabetes; high fructose;STZ; oxidative stress.

Abstract

The objective of this study was to evaluate the possible antidiabetic and insulin tropic effect of nigella sativa seeds water extract and/or lipoic acid. For this purpose, Forty male Wistar strain albino rats were divided into five groups (8 rat /group), group 1; control group, group2; normal rats fed on basal diet and received 21% fructose in drinking water for four weeks then injected intraperitoneally by a single streptozotocin (STZ) dose (40 mg/kg body weight)to induce type2 diabetes and served as diabetic control group, group3; diabetic rats orally administrated water extract of nigella sativa (1ml/kg daily for 6 weeks), group4; diabetic rats orally administrated lipoic acid (100 mg/kg body weight daily for 6 weeks), group5;diabetic rats orally administrated aqueous extract of nigella sativa and lipoic acid daily simultaneously at the previous tested doses for 6 weeks. Untreated diabetic group showed a significant increase in serum glucose, total cholesterol, triacylglycerols, transforming growth factor beta (TGF-β)and leptin. Also, hepatic total lipids, total cholesterol, alcohol dehydrogenase (AD) activity were significantly elevated in untreated diabetic group as compared to normal control. Whereas, serum insulin, C-peptide, insulin like growth factor -1(IGF-1), hepatic glycogen and NADPH were significantly reduced in untreated diabetic group as compared to normal control. Moreover, the activities of hepatic enzymes cytochrome p450 reductase, glucose-6-phosphate dehydrogenase (G-6-P-D), Glyoxalase I and II were significantly reduced in untreated diabetic rat as compared to normal control group ($p<0.05$).Oral administration of nigella sativa seeds water extract and /or lipoic acid restored all these altered biochemical parameters to near normal levels.

Conclusion the ameliorative effect of water extract of nigella sativa and /or lipoic acid on altered carbohydrate, lipid and oxidative stress variables in type 2 diabetic rats, may be attributed to their insulin releasing capacity, lipid lowering effect and antioxidative effect.

Introduction

Diabetes affects hundreds millions people worldwide. The prevalence of diabetes has increased dramatically during the last two decades and it is expected to increase further, probably due to increased consumption of high calorie , high fat diets and sedentary life style (Ramachandran,2005). Between two major types of diabetes, type 2 is the most prevalent in adult with different range of age and gradually increasing in young children and adolescents (WHO,2010).Diabetes mellitus is a multi-faceted metabolic disorder where there is increased oxidative stress that contributes to the pathogenesis of this debilitating disease (Golbidi et al.,2011).This has prompted several investigations into the use of antioxidants as a complementary therapeutic approach.

Some plants have been in use in many Middle Eastern countries as a natural remedy for diabetes in traditional medicine; nigella sativa is one of these plants (Javanbakhat et al., 2013). The pharmacological action and potential therapeutic activities of nigella sativa have been proved for the management of many other disease such as

bronchitis, immune disease, bacterial infection, hypertension, liver disease, gastrointestinal disease and allergic condition (Kanter, 2007). Many studies have also showed that the extract from the seeds of nigella sativa have antidiabetic, antioxidant, antihyperlipidemic effect (Sheikh et al., 2013 and Salama et al., 2011)

Alpha lipoic acid, a naturally occurring dithiol compound which play an essential role in mitochondrial bioenergetics reactions, has gained considerable attention as antioxidant for use in managing diabetic complications (Golbidi et al., 2011).In experimental animals, Alpha lipoic acid restored insulin, stimulated glucose uptake into skeletal muscles, and as a result may act as a hypoglycemic agent through basal and insulin-activated glucose uptake (Moini et al., 2002). Additionally, It has been shown to improve glucose metabolism in diabetic patients (Bitar et al., 2014).Therefore, the present study was undertaken to investigate the possible antidiabetic and insulintropic effect of nigella sativa seeds water extract and/or lipoic acid in type 2 diabetic rats.

Materials and methods

Materials

Chemicals:
Fructose was purchased from International Company for Scientific and Medical Supplies. Cairo, Egypt. Nigella sativa was purchased from Faculty of Agriculture, Cairo University, Giza, Egypt. Kits for measurement of glucose and lipidprofiles were purchased from Diagnosticum Zrt, Budapest, Attila and those for NADPH and glycogen were purchased from Genway Biotech, INC,USA. Insulin ultrasensitive immunoassay kits was obtained from, Holzel Diagnostika, Germany. C-peptide enzyme –linked immunosorbent assay (ELIZA) kits was obtained from Cosmo Bio Co., LTD, Tokyo, Japan. Glyoxalase activity kits were obtained from Sigma-Aldrich, USA. Transforming Growth Factor beta (TGF-β) ELIZA kits was obtained from Kamiya Biomedical Company (Cat.No.KT-30309).Insulin growth factor -1(IGF-1) and leptin ELIZA kits were obtained from IBL, America. NADPH cytochrome p450 reducatse and Orexins-1 and 2 ELIZA kits were obtained from USCN, Life Science Inc. All other chemicals were of analytical grade.

Animals:
Forty adult male, Wistar strain albino rats weighing (200g ±5 g) were purchased from breeding unit of Medical Research Center (Faculty of Medicine, Ain Shams University). The rats were housed in individually under controlled condition of temperature (25C ±5 C) humidity (50% ±10%), and acclimatized to 12 hr light/dark .The experimental period was 6 weeks on which food and water were provided ad libitum. Animal's experiment was conducted according to the guidelines of institutional animal ethical committee.
Diet:Basal diet was based on AIN-93 recommendations Reeves et al.(1993)

Methods:
Preparation of aqueous extract of nigella sativa seeds:
An extract of nigella sativa seeds was prepared using the method of Mohammed (2012). The seeds were crushed manually in a mortar with a pestle. A volume of 100 ml of distilled water was added to 20 g of dry powder. It was vortexed continuously until there was no further change in color of the solution. This solution was centrifuged for 15 min. The supernatant (brownish-orange in color) was filtered through Whatman filter No.1using Buchner funnel and stored at 4 °C in sterile tubes until use.

Induction of Diabetes:
All animals were acclimatized for one week before onset of experiment in laboratory. Animals of diabetic groups (2,3,4 and 5) were fed on basal diet and received 21% fructose for 4 weeks (21% fructose) in drinking water then injected intraperitoneally by a single streptozotocin (STZ) dose (40 mg/kg) (Kumar et al .,2014) to induce type 2 diabetes (fructose fed/STZ diabetic model). The animals were fasted for 12 hours before STZ injection. STZ was freshly prepared in 0.1 Mcitrate phosphate buffer (pH 4.5)

Experimental design:
The animals were randomly assigned into five experimental groups (each of 8). All international and local rules and regulation for handling animals in experiments were followed. The experimental groups illustrated as follows:
Group1: normal rats fed on basal diet and served as normal controls.
Group2: normal rats fed on basal diet and received 21% fructose for 4 weeks then injected by STZ intraperitoneally (40mg/kg body weight) ***(Kumar et al.,2014)*** and served as diabetic control group.
Group3: diabetic rats received aqueous extract nigella sativa (1ml/kg body weight) daily by intragastric tube for 6 weeks (equivalent to 200mg/kg body weight P.O) ***(Hasan et al.,2014)***
Group 4: diabetic rats received lipoic acid (100mg/kg body weight) daily by intragastric tube for 6weeks ***(Chun et al.,2009)***
Group 5: diabetic rats received aqueous extract of nigella sativa and lipoic acid daily simultaneously at the previous tested doses for 6 weeks.

At the end of the experiment, the animals were anesthetized with diethyl ether after 12 hours fasting and whole blood samples were taken from hepatic portal vein. The blood samples left for 15 minutes at 37°C for serum separation, then centrifuged at 3000 rpm for 20 minutes, then sera were separated and kept in plastic vials at −20°C until analyses.

Preparation of liver homogenate:
The liver of the rats from individual group was dissected out, washed with ice-cold saline and weighed each sample were divided into 3 parts. First part was homogenized in 50 mM phosphate buffer (pH 7.4) using an electronic homogenizer to prepare 10% w/v homogenate (pH 7.4). The homogenate was centrifuged at 3000 rpm g for 30 min. The supernatants were used for measuring hepatic glucose-6-phosphate dehydrogenase (G-6-P dehydrogenase) activity according to method of ***Lohr and Waller (1974)*** and Cytochrome p450 reductase was analyzed using (ELIZA) according to the manufacturer's instructions. NADPH level, Glyoxalase I, glyoxalase II and alcohol dehydrogenase (AD) activities were determined according to quantitative colorimetric method of ***Kupfer and Munsell (1968), Thornalley,(1993), Martins et al.(1982) and Vallee and Hoch (1955),*** respectively. Second part was homogenized with a mixture of chloroform and methanol, for extraction of total lipids and total cholesterol. The supernatants were used for measuring hepatic total lipids and total cholesterol according to the method of ***Blig and Dyer (1959) and Richmond (1973)***. The third part was homogenized in 3 volumes of 0.25 M sucrose containing 0.001 M EDTA. The homogenate was then centrifuged. The supernatant fluid obtained after 10 minutes is referred to as “crude extract” Which used for glycogen quantitative colorimetric method of ***Dalrymple and Hamm (1973).***

Biochemical assays:
Serum glucose was estimated using oxidase peroxidase according to the methods of ***Trinder (1969),*** serum rat insulin was estimated by ultrasensitive rat insulin ELIZA according to the method of ***Finlay and Dillard (2007)***, C-peptide, IGF-1and TGF-βlevels were analyzed by ELIZA according to the method of ***Massey and Smyth (1975), Rinderknet and Humbel (1978)*** and ***Javelaud and Mauviel (2004)*** following manufacturer's instructions . Serum total cholesterol and triglycerides were determined according to the quantitative colorimetric method of ***(Richmond,1973)*** and ***Fassati and Prencipe (1982)***. Leptin was determined according to Radio-immunoassay assay according to the method of ***(Zhongminet al.,1996)*** .Orexin-1and Orexin-2 were analyzed using ELIZA according to the method of ***Porkka-Heiskanen et al.(2000)*** following the manufacturer's instructions

Statistical Analysis:
The data were presented as means ±SD. One way analysis of variance (ANOVA) followed by post- hoc least significant difference analysis (LSD) at ($p< 0.05$) was performed using the statistical package for social science (SPSS) version 9 to compare all treated groups. Differences were considered to be significant when ($p<0.05$).

Results

Table (1): Serum glucose, insulin, C-peptide, IGF-1 and hepatic glycogen in different experimental groups

Parameters	Group1	Group2	Group3	Group4	Group5
Glucose (mg/dl)	117.33± 3.5	248 ± 91[a]	165 ± 72[a,b]	202.66 ± 9.5[a,b,c]	143.33 ± 8.02[a,b,c,d]
Insulin (µmol/L))	1.66 ± 0.15	0.73 ± 0.11[a]	1.33 ± 1.1[a,b]	1.03 ± 0.11[a,b,c]	1.4 ± 0.01[a,b,c,d]
C-peptide (ng/ml))	0.733 ± 4.04	0.29± 7.7[a]	0.536 ± 2.5[a,b]	0.426 ± 3.5[a,b,c]	0.65 ± 4.5[b,c,d]
IGF-1 (ng/ml)	500.6 ± 15.3	276.33 ± 8.02[a]	381 ± 9.5[a,b]	337 ± 9.5[a,b,c]	436 ± 2.3[a,b,c,d]
Glycogen content (µg)	7.5 ± 0.26	6.63 ± 0.15[a]	7.73 ± 0.28 [b]	7.06 ± 0.15[a,b]	7.63 ± 0.208[b,d]

Group1: control group, Group2: diabetic control group, Group3: diabetic rats treated with nigella sativa ,Group 4: diabetic rats treated with lipoic acid , Group 5: Diabetic rats treated with nigella sativa and lipoic acid Significant difference (P < 0.05): (a) compared to group 1, (b) compared to group 2, (c) compared to group 3, (d)compared to group 4.

Table (2): Serum leptin, Orexin-1, Orexin-2 and TGF-B in different experimental groups

Parameters	Group1	Group2	Group3	Group4	Group5
Leptin (pg/ml)	5.033±0.282	7.76 ± 0.115[a]	6.8 ± 0.1[a,b]	7.23 ± 0.21[a,b,c]	5.86 ± 0.025[a,b,c,d]
Orexin-1(pg/ml)	28.43 ± 1.45	14.76 ± 1.30[a]	18.83± 0.55[a,b]	16.93 ± 0.92[a,b,c]	23.8 ± 1.3[a,b,c,d]
Orexin-2 (pg/ml)	37.16 ± 3.9	39.54± 3.76[a]	38.89 ± 3.54	28.44 ± 4.01	31.70 ± 5.53
TGF-β1 (pg/ml)	26.63 ± 1.45	63.8 ± 2.88[a]	41.2 ± 3.7[a,b]	53.3 ± 1.5[a,b,c]	37 ± 1.27[a,b, d]

As legend in table (1)

Table (3): Serum total cholesterol, serum triacylglycerols, hepatic total lipids and total cholesterol in different experimental groups

Parameters	Group1	Group2	Group3	Group4	Group5
Serum total cholesterol (mg/dl)	56.63±1.06	94.73 ± 2.63[a]	73.76 ± 3.3[a,b]	80.86 ± 1.51[a,b,c]	64.76± 1.66[a,b,c,d]
Serum triacylglycerols (mg/dl)	62.13 ± 1.8	121.83 ± 5.3[a]	92.03± 4.07[a,b]	102.3 ± 3.3[a,b,c]	85.2±3.47. [a,b,c,d]
Hepatic total lipids (mg/g)	40.06 ± 0.65	79.76± 1.37a	57.7 ± 2.35[a,b]	66.7 ± 1.9[a,b,c]	46.06 ± 1.35[a,b,c,d]
Hepatic total cholesterol(mg/g)	2.46± 0.11	4.33 ± 0.14[a]	3.47 ± 0.02[a,b]	3.74 ± 0.1[a,b,c]	2.95 ± 0.13[a,b,c,d]

As legend in table (1)

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Table (4): Hepatic NADPH level , Glucose-6-phosphate dehydrogenase , cytochrome p450 reducatse, glyoxalase I, glyoxylase II and AD activities in different experimental groups

Parameters	Group1	Group2	Group3	Group4	Group5
Cytochrome(P450) Reductase(ng/ml)	1.82 ± 6.02	1.03 ± 6.02[a]	6.8 ± 0.1[a,b]	1.36± 0.1[a,b,c]	1.66 ± 5.5. [a,b,c,d]
Glucose-6-phosphate dehydrogenase (µmol/min/mg protein)	2.77 ± 0.5	1.51 ± 0.09[a]	2.06± 0.1[a,b]	1.74 ± 0.02[a,b,c]	2.44± 0.02[a,b,c,d]
NADPH (ng/mg protein)	1.33± 0.45	0.66± .0.28[a]	0.91 ± 0.13 [a,b]	0.80 ± 0.25 [a,b,c]	1.13 ± 7.02 [a,b,c,d]
Glyoxalase I (µmol/min/mg protein)	13.86 ± 0.25	7.3 ± 0.88[a]	10.86 ± 0.45[a,b].	9.93 ± 0.55[a,b,c]	12.8±0.35 [a,b,c,d]
Glycoxalase II (µg of GSH consumed/min/mg	5.1 ± 0.26	2.66± 0.25[a]	3.76 ± 0.25[a,b]	3.53 ± 0.2 [a,b,c]	4.43 ± 0.25[a,b,c,d]
AD (nmol/min/ml)	24.66 ± 0.94	60.8 ± 2.1 [a]	47.81 ± 2.17[a,b]	52.15 ± 1.98[a,b,c]	30.3± 1.93[a,b,c,d]

As legend in table (1)

The results summarized in table (1) showed that there was a significant increase in serum glucose level in untreated diabetic group as compared to normal control group($p<0.05$).This increase in serum glucose level was accompanied by a significant reduction in serum C-peptide , insulin and IGF-1 levels and hepatic glycogen content. Oral administration of nigella sativa water extract and /or lipoic acid showed a significant improvement in these altered levels. The results reported in table (2) showed that serum leptin and TGF-β1 significantly increased in untreated diabetic group as compared with normal control group ($p<0.05$).The increment ratio in serum leptin and TGF-β1 of diabetic group were 54% and139 % as compared with normal control group. The increase in serum leptin and TGF-β1 levels were accompanied by a significant decrease in serum orexin-1. Treatment with nigella sativa seeds water extract and /or lipoic acid resulted in a significant improvement in these altered levels. Concerning the orexin-2 level there was no significant difference between different experimental groups.

From the results reported in table (3) it is clear that diabetes induction resulted in a significant elevation in hepatic total lipid , hepatic total cholesterol , serum triglycerides and serum total cholesterol by 67.27%,96%,99% and76%, respectively as compared with normal control ($p<0.05$). Treatment with nigella sativa water extract and /or lipoic acid caused a significant decrease in hepatic and serum altered lipids parameters.

Results reported in table (4) showed that hepatic G-6-P dehydrogenase activity and NADPH level was significantly decreased in untreated diabetic group as compared with normal control group ($p<0.05$). Oral administration with nigella sativa water extract and /or lipoic acid caused significant increase in G-6-P-D activity with a subsequent increase in NADPH levels.

Hepatic cytochrome p450 reducatse, glyoxalase I and glyoxalase II activities were significantly reduced in the liver homogenate of untreated diabetic group as compared with normal control group ($p<0.05$). On the other hand, the activities of hepatic AD was significantly increased in untreated diabetic group as compared with normal control group. Treatment with nigella sativa water extract and /or with lipoic acid caused a marked decrease in hepatic AD but the decrease was more evident in diabetic group treated with nigella sativa and lipoic acid in combination.

Discussion

The pathogenesis of type 2 diabetes in an animal model is most likely similar to the pathogenesis in human. Thus, this study was initiated by developing a suitable type 2 diabetes in rat model that is closely mimic the natural history of human type 2 diabetes from insulin resistance to beta dysfunction. This was achieved by feeding high

fructose diet for 4 weeks which induce insulin resistance followed by intraperitoneal injection with a single low dose of STZ that cause initial beta dysfunction.

The results of the present study showed that fructose fed /STZ diabetic rats model (type 2 model) exhibit a significantly elevated fasting serum glucose which accompanied by diminished serum insulin, C-peptide, IGF-1 levels and hepatic glycogen content. Similar results obtained by ***Kumar et al.(2014) and Islam et al.(2001)*** who used fructose fed /STZ injected model for type2 diabetes induction. The present data also demonstrated that the treatment of diabetic rats with aqueous extract of nigella sativa and /or lipoic acid caused a potential amelioration of serum glucose elevation. These results suggested an increase in endogenous insulin secretion in case of diabetic rats treated with aqueous extract of nigella sativa and /or lipoic acid which might be the cause of its hypoglycemic effect. The significant reduction in elevated serum glucose levels is supported by the previous results of ***Ali-Mohammed et al. (2013)*** reported that the hypoglycemic effect of nigella sativa extract by diminishing the fasting blood glucose level in STZ diabetic rats. Also, ***Sheikh et al.(2013)*** reported that nigella sativa significantly reduced the hyperglycemia in type2 diabetic rats.

Evaluation of C-peptide level is useful parameter to indicate the amount of endogenous insulin secreted in the body (***Marques et al.,2004)***. The results of the current study were supported by the previous study of ***Salama (2011)*** who reported alpha lipoic acid or nigella sativa water extract showed a significant increase in serum insulin and C-peptide levels. Another study of ***Benhaddou et al.(2010)*** reported that the antidiabetic effect of nigella sativa may be due to its stimulatory effect on beta cell function with consequence increase in serum insulin level. Similar results obtained by ***Zhang et al.(2009)*** who reported that lipoic acid is referred to as an insulin mimetic agent hence, it is a potential new drug. In the same line, ***Singh et al.(2008)***confirmed that lipoic acid speeds the removal of glucose from the blood stream at least partly by enhancing insulin function and reduced insulin resistence.

The reduction in hepatic glycogen content in fructose fed /STZ diabetic rats as compared to normal control group are in harmony with those of ***Ahmed et al.(2010) and (2011)*** who found that STZ-induced diabetes reduced hepatic glycogen content. This change is obviously due to insulin deficiency, which in turn results in the activation of glycogenolytic and gluconeogenic pathways (***Raju et al.,2001).*** The elevation of liver glycogen content after treatment with aqueous extract of nigella sativa and /or lipoic acid may be due to amelioration of altered enzyme activities secondary to the increase of insulin level in the blood.

IGF-1 has similar structures and functions like those of insulin particularly for peripheral uptake of glucose and fatty acids (***Teppala and Shankar,2010).*** IGF-1 may probably be involved in metabolic abnormality and with diabetes .The results of the current study showed that there were a significant reduction in serum IGF-1 in untreated diabetic rats as compared with normal control group. Whereas, Oral administration with nigella sativa seeds water extract and/or lipoic acid significantly improved serum IGF-1 levels near normal levels. Our results are supported by ***Li et al.(2004)*** who reported that serum IGF-1 levels were significantly decreased in diabetic rats.

High flux of fructose to the liver leads to a significantly enhanced rate of de novo lipogenesis and triacylglycerols synthesis, driven by the high flux of glycerol and acyl portions of triacylglycerols molecules from fructose catabolism. Stimulated triacylglycerols synthesis is likely lead to hepatic accumulation of triacylglycerols (***Basciano et al., 2005).*** As shown in table (3), HFD / STZ diabetic rats exhibited abnormalities in lipid metabolism generally led to a significant elevation in the levels of serum and hepatic lipids. Oral administration of aqueous extract of nigella sativa an /or lipoic acid produced remarkable amelioration of lipogenthesis, which reflected in the significant improvement in serum and hepatic altered lipid parameters. These results are similar to the results of ***Kaleem et al.(2006)*** who reported oral administration of nigella sativa water extract significantly reduced serum total cholesterol and triacylglycerols.

Orexins- 1 and 2 are novel pairs of neuropeptide that appear to play a role in the regulation of energy homeostasis ***(Beek et al.,2006).*** The results shown in table (2) reported that induction of type 2 diabetes resulted in a significant decrease in orexin-1 levels as compared to normal control group. This decrease in orexin A in untreated diabetic group was accompanied with a significant increase in serum leptin. With respect to orexin B

levels, there was no significant difference between different experimental groups. In the same line, ***Ibrahim et al.(2006)*** confirmed that obesity and type 2 diabetes are associated with decreased orexin-1 level and increased leptin concentration. This inverse relationship between the two peptides suggests a tight link between them in control of energy homeostasis rather than food intake under both physiological and pathological conditions. Similar results obtained by ***Heibashy et al.(2010)*** who reported that the inverse relationship between leptin and both insulin and orexin levels in obese and type 2 diabetic rats.

TGF-β1 is an important cytokine for the development of renal injury in type 2 diabetic patients (***Le et al.,2005)***. The results of the current study showed that , there were a significant increase in serum TGF-β1in untreated diabetic group as compared to normal control group ($p<0.05$). The results also showed that oral administration of nigella sativa seeds water extract and /or lipoic acid resulted in a significant decrease in serum TGF-β1 as compared to untreated diabetic group ($p<0.05$). In a previous study of ***Yener et al .(2007)*** the results demonstrated that TGF-β1 levels are elevated in women with a history of gestational diabetes mellitus. Also, our results are in agreement with the results of ***Yener et al.(2008)*** showed that serum TGF-β1 positively correlated with hyperglycemia.

The glyoxalase system consists of 2 enzymes, glyoxalase I and glyoxalase II. Glyoxalase I catalyses the formation of S-D-lactoglutathione while glyoxalase II catalyses the hydrolysis of S-D lactoylglutathione to D -lactic acid and GSH ***(Vanithadevi and Anuradha,2008)***. Regarding the data in table (3), untreated diabetic group showed a significant decrease in the glyoxalases I and glyoxalase II activities as compared to their corresponding control group. This may be attributed to consumption of high fructose diet which metabolized through glycolytic pathway producing excess methylglyoxal and aldehyde. These products can in turn activate the stress response pathway. Oral administration of lipoic acid and /or aqueous extract of nigella sativa separately or in combination has been found to reverse the activities of glyoxalases I and glyoxalase II to normal values. These results are in accordance with those reported by ***Pooranaperundevi et al.(2010) and Heibashy et al.(2013)*** who reported the decreased activity of glyoxalase I and II in experimentally induced diabetes in rats.

The results in table (4) also demonstrated that there was a significant decrease in hepatic NADPH level, G-6-P dehydrogenase and cytochrome p450 reductase activities in diabetic rats as compared to normal control group.

G-6-P dehydrogenase is the principal source of the major intracellular reductant NADPH, which is required by many enzymes, including enzymes of antioxidant pathway ***(Zhang et al.,2010***). Therefore, a significant decrease in G-6-P dehydrogenase activity led to a subsequent reduction in NADPH levels and make cell sensitive to oxidant damage (***Leopold et al.,2003***). Also, the activities of NADPH requiring enzymes as cytochrome p450 reductase could be affected by depletion of NADPH. Our results showed that oral administration of aqueous extract of nigella sativa and/or lipoic acid reduced glucose concentration, ameliorated oxidative stress and normalized the activity of G-6-P-D, thus restoring NADPH levels and cytochrome p450 reductase activity in liver.

The results of the present study reported that there was a significant elevation in AD activity in liver homogenate of diabetic rats as compared to normal control rats. This increase may be attributed to the induction of polyol pathway in which aldose reductase sitmulate the conversion of aldose sugars to their respective alcohols at the expense of NADPH (***Srivastava et al.,2000***). Similar results obtained by ***Neidowicz and Daleke(2005***) who reported that an increase in aldose reductase activity could harm cells by NADPH depletion and alcohol accumulation.

The hypoglycaemic mechanism of nigella sativa, which is apparent in this study may be either through hepatic gluconeogenesis ***(Fararh et al.2004***) .Extra-pancreatic mode of nigella sativa action can be explained by the presence of insulin -like peptides or glucose absorption inhibitory potentials of nigella sativa (***Meddah et al.,2009)***.Furthermore, ***Abdelmeguide et al.(2010)*** reported that the antihyperglycemic effect of nigella sativa oil and its active component thymoquinone could be due to reduction in oxidative stress, thus preserving pancreatic B-cell integrity lead to an increase in insulin level. Finally, the hypoglycemic mechanism of nigella sativa was at least partly, from a stimulatory effect on beta cell function with consequent increase in serum insulin level which possesses insulintropic properties in type 2 diabetic rats (***Fararh et al.,2002***).

The mechanism by which alpha lipoic acid supplementation exerts the antidiabetic effect through its antioxidant properties. Also, lipoic acid is an insulin mimetic; hence, it is a potential new antidiabetic agent ***(Zhang et al.,2009)*** . Besides that, alpha lipoic acid supplementation also effectively inhibits dyslipidemia and improved hyperglycemia condition (***Budin et al.,2009).***

The results of the current study reported that the improvement in most altered parameters was evident in diabetic rats treated with nigella sativa and lipoic acid in combination, suggesting that there is a synergistic effect between lipoic acid and nigella sativa seeds water extract in diabetes treatment.

Conclusion

From the results of the current study ,it can be concluded that the ameliorative effect of water extract of nigella sativa and /or lipoic acid on altered carbohydrate , lipid and oxidative stress variables in type 2 diabetic rats may be attributed to their insulin releasing capacity, lipid lowering effect and antioxidative effect.

References

1. Ahmed,M.o., Mohamed,A.M, Abdel-Moneim,A and Ashour,B(2011): Antidiabetic effect of hesperidins and naringin in type2 diabetic rats . Diabetol.Croat.,41(2):51-67.
2. .Ahmed,M.O.,Abdel-Moneim,A.,Abulyzid,I and Mohamed,A.M(2010): AntihyperlipidemicAntihyperglycemic,Antihyperlipidemicand antioxidants effects and the probable mecanisms of action of Rutagraveolens and rutin in nictotinamide/STZ diabeticalbinorats.Diabetol.Croat., 39:15-32.
3. Ali-Mohamdi,S.,Hobbenaghi,R.,Javanbakhat,J.,Khenderadmad,D.,Mortezaee,R., Tavakoli,M., Khadivar,f.,andAkbari,H (2013): Protective and Antidiabetic effects of extract from nigella sativa on blood glucose concentration against STZ- induced diabetic rats : an experimental study with histopathologicalevalutation. Diagnostic Pathology., 8:137
4. Abdelmeguide,N.E.,Fakhoury,R.,Kamal,S.M and Al-wafi,R.J.(2010): Effects of nigella sativa and thymoquinone on biochemical and subcellular changes in pancreatic B-cells of streptozotocin-induceddiabetic rats .Diabetes., 2(4):256-66
5. Basciano,H.,Federico.L and Adeli,K (2005):Fructose ,insulin resistence and metabolic dyslipidemia. Nutrmetabolism ., doi:10.1189/1743-7075-2-5
6. Beek,B.,Kozak,R.,Moar,K.M and Mereer,J.G(2006): Hypothalamic orexigenic peptide are overexpressedin young long-Evans rats after early life exposure to fat richdiets.Biochem. Biophys.Res.Commun.,342(2):452-8
7. Benhaddou,A.,Martineau.L.C.,Vallerand,D.,Haddad,Y.,Afahar,A.,Settaf,A.,Haddad,P.S (2010): Multiple molecular targets underlie the antidiabetic effect of Nigella sativa seeds extract in skeletal muscle, a dipocyte and liver cells .Diabetes ObesMetab ;12(2):148-57
8. Bitar, M. S., Wahid,S., Pilcher,C.W.T., Al-saleh,E and Al-Mulla, F (2014): Alpha lipoic acid mitigates insulin resistence in Goto-Kakizakirats.HormMetab Res.,36:542-549
9. Bligh,E.G and Dyer,W.J.(1959) : A rapid method for total lipid extraction and purification. Can.J.Biochem.Physiol., 37:911-917
10. Budin,S.B., Othaman,F., Louis,S.R., Bakar.M.A and Radzi,M.,Osman,K .,Das,s.,Mohamed,J (2009): Effect of alpha lipoic acid on oxidative stress and vascular wall of diabetic rats. Romanian Journal of Morphology and Embryology.,50(1):23–30
11. Chan-jun,L.,Qiu-mei,Z.,Ming-zhen,L.,Jin-yun,Z.,Pei.Y.,De-min.Y(2009): Attenuation of myocardial apoptosis by alpha-lipoic acid through suppression of mitochondrial oxidative stress to reduce diabetic cardiomyopathy.Chin. Med. J., 122(21):2850-2586
12. Dalrymple, R. H. and Hamm, R: (1973) A method for the extraction of glycogen and metabolites from a single muscle sample. Intl. J. Food Sci& Tech.,8(4): 439-444.

13. Fararh, K.M., Atoji, Y., Shimizu, Y., Takewaki, T (2002): insulinotropic properties of Nigella sativa oil in Streptozotocin plus Nicotinamide diabetic hamster. Rec. Veter. Sci.,73 :279-82.
14. Fararh, K.M., Atoji ,Y., Shimizu, Y (2004): Mechanisms of the hypoglycaemic and immunopotentiating effects of Nigella sativa L. oil in streptozotocininduced diabetic hamsters. Res Vet Sci., 77: 123-9. 24.
15. Fassati, P and Prencipe, L. (1982): Serum triglycerides determined in colorimetrically with an enzyme that produce hydrogen peroxide. Clin. Chem., 28: 2077.
16. Finlay, J.W.A., Dillard, R.F(2007): Appropriate Calibration Curve Fitting in Ligand Binding Assays. AAPS Journal., 9(2): E260-E267.
17. Golbidi,S.,Badran,M and Laher,I (2011): Diabetes and alpha lipoic acidForntier in Pharmacology.,2(69):1-13
18. Hasan,M.N.,Khan,R.A.,Nassiruddin,M and Khan,A.A (2014):Protective effect of nigella sativa against aspirin induced gastric damage in rats . Int J Pharm Pharm Sci., 6(5): 275-278
19. Heibashy,M.I.A.,Mazen,G.M.A and Kelada.N.A.H (2013):Insulin Resistence induced by high fructose diet in rats due to hepatic disturbance.Arab J. Nuc. Sci. Appl.,46(3):297-306
20. Heibashy,M.I.A.,Zaki,N.A and El-Nahrawy.W.A.M (2010): Alteration in insulin ,leptin,Orexin-A and Neuropeptide Y levels in high-fat diet rats. Aust. J. Basic & Appl. Sci.,.,4(6):1473-1482
21. Ibrahim,H.S.,El Ghonty,S.S.,Morsy,E.y and Soliman ,E.M.(2006): Role of orexin-A in obesity and type2 diabetes in male patients and its correlation with leptin hormone.Bull.Alex.Fac.Med.,42(3):825
22. Javelaud, D and Mauviel, A (2004) : Mammalian transforming growth factor-beta: Smad signaling and physio-pathological roles. Int J Biochem Cell Biol ., 36 : 1161-5
23. Javanbakhat ,J.,Hobbenaghhi,K.,Hosseini,E.,Bahrami,A.M.,Khadivar,F.,Fathi,s and Hassan,A.M (2013):Histopathological investigation of neuroprotective effect of nigella sativa on motoer neuron anterior horn spinal cord after sciatic nerve crush in rats .Pathol.Biol.doi:10.1016
24. Kaleem,M.,Kirmani,D.,Asif,M.,Ahmed,Q and Banco,B (2006): Biochemical effects of nigella sativa L-seeds in diabetic rats . Indian J of Exp Biology.,44: 745-748.
25. Kanter, M (2007). Effects of Nigella sativa and its Major Constituent, Thymoquinone on Sciatic Nerves in Experimental Diabetic Neuropathy.Neuroche Res.,23 :87-96.
26. Kumar,C.,Kumar,R and Nehar,s.(2014): Induction of type 2 diabetes by high –fructose diet and low dose of intraperioneal injection of STZ in albion rats .International journal for pharmaceutical reseasech scholars.,3(1): 196-202
27. Kupfer,D andMunsell,T(1968): A colorimetric method for the quantitative determination of reduced pyridine nucleotides (NADPH and NADH). Anal. Biochem.,25:10-16
28. Le,y.,Yu., X.andRuas.L (2005): the immunopharmacological properties of transforming growth beta.Int,immunopharmacol.,5:1771-1782.
29. Leopold, J.A., Zhang. ,Y.Y., Scribner, A.W., Stanton, R.C., Loscalzo, J. (2003): Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide.ArteriosclerThrombVasc Biol;23:411–417
30. Li, J.B., Wang ,C.Y., Chen ,J.W, Feng, Z.Q, Ma, H.T (2004): Expression of liver insulin-like growth factor 1 gene and its serum level in patients with diabetes.World J. Gastroenterol.,10(2):255-9.
31. Lohr G.W., Waller, H.D (1974): Glucose-6-Phosphate Dehydrogenase. Methods of Enzymatic Analysis,(3rd Edition)VarlagChemie, Wehenheim., 3:636.
32. Massey, D. E. and Smyth, D. G. (1975) Guinea pig proinsulin. Primary structure of the C-peptide isolated from pancreas. J. Biol. Chem. 250., 6288-6290.
33. Marques,R.G.,Fontaine,M.J.,Roqes,J (2004):C-peptide: much more than a byproduct of insulin biosynthesis.Pancreas.,29(3):231-8
34. Martins AM, Cordeiro C & Freire AP (1999) Glyoxalase II in Saccharomyces cerevisiae: in situ kinetics using the 5,5′-dithiobis (2-nitrobenzoic acid) assay. Arch BiochemBiophys 366, 15–20.DO
35. Meddah, B., Ducroc, R., El-Abbes Faouzi, M(2009): Nigella sativa inhibits intestinal glucose absorption and improves glucose tolerance in rats. J Ethnopharmacol., 121:419-24
36. Mohammed, N.A(2012): Effect of Nigella Sativa Basic Sciences 154 Effect of Nigella Sativa L. extracts against Streptococcus mutans and Streptococcus mitis in Vitro, J BaghColl Dentistry.,24(3):154-157

37. Moini, H., Packer, L., and Saris, N. E. (2002): Antioxidant and prooxidant activities of alpha-lipoic acid and dihydrolipoicacid.Toxicol. Appl. Pharmacol., 182: 84–90.
38. Niedowicz , D.M and Daleke,D.M (2005): The Role of Oxidative Stress in Diabetic Complications.CellBiochem Biophysics .,43: 289-330
39. Pooranaperundevi,M.,Sumiyabanu,M.,Viswanathan,R.,Sundarapandiyan,R., Anuradha,c (2010) :Insulin resistance induced by a high-fructose diet potentiates thioacetamide hepatotoxicity .Singapore.Med.J.,51(5):389.
40. Porkka-Heiskanen,T.,Alanko,L., Kalinchuk,A., Heiskanen, S and Stenberg,D (2000): Plasma orexin A and B in obese women.Neuroendocrinology Lett.,22(1):128-134
41. Raju,J.,Gupta,D.,Rao,A.R.,Yadava,P.K.,Baquer,N.Z (2001): TrigonellaFornumGraecum seed powder improve glucose homeostasis in alloxan diabetic rats tissues by reversing the altered glycolytic,glyconeogenic and lipogenic enzymes .Mol.Cell.Biochem.,224:45-51
42. Ramachandran,A.(2005): Epidemology of diabetes in indiathress decades of research.JAssoc Physicians India. , 53:34-8.
43. Reeves, G.; Nielsen, F.H. and Fahey J.R.G.C. (1993): AIN-1993 reformation of the AIN-76: purified diet for laboratory rodents: Final Report of The American Institute of Nutrition, purified AdHOC writing committee on the Reformation of the AIN-76 A rodent diet. American Institute., J. Nutr. 26: 1939-1951.
44. 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. 19: 1350.
45. Rinderknech,E and Humbel, R.E.(1978): The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin . J Biol Chem. 25;253(8):2769-76
46. Salama,R.H.M (2011):Hypoglycemic effects of lipoic ,Carnitine and nigella sativa in diabetic rats model.International journal of health science.,Qassium university.,5(2):126-134
47. Sheikh,T.J.,Joshi,D.V.,Patel,B.J and Modi,C.M (2013): Protective role of nigella sativa against experimentally induced type 2 diabetic nuclear damage in Wistar rats . Vet World. .,6(9):698-702.
48. Srivastava, S., Dixit, B. L., Cai, J., Sharma, S., Hurst, H. E., Bhatnagar,A. (2000) Metabolism of lipid peroxidation product, 4- hydroxynonenal (HNE) in rat erythrocytes: role of aldose reductase. Free Radic. Biol. Med., 29: 642–651.
49. Teppala, S.,Shankar, A.(2010): Association between serum IGF-1 and diabetes among U.S. adults.DiabetesCare., 33(10):2257-9.
50. Trinder, P. (1969): Determination of glucose by enzymatic colorimetric method. Ann. Clin. Biochem., 6: 24.
51. Thornalley ,P.J(1993): The glyoxalase system in health and disease. Mol Aspects Med., 14(4):287-371.
52. Vallee, B., and Hoch, F., "Zinc (1955): A Component of Yeast Alcohol Dehydrogenase". Proc. Natl. Acad. Sci. USA, ., 41, 327 .
53. Vanithadevi,B.andAnuradha,C.V.(2008): Effect of rosmarinic acid on insulin sensitivity, glyoxalase system and oxidative events in liver of fructose-fed mice.Int J Diabetes and Metabolism.,16: 35-44 35
54. World Health organization (2010):Facts and figures about diabetes.http://www.WHO.int/diabetes/facts/en/Acessed 5 dec 2011
55. Yener,S.,Comlekci,A.,Akinci,B.,Akan,p.,Dermir.T.,Bayrakatar,F and Yesil,S(2008): Serum transforming growth factor-beta-1 in normoalbuminuric and normotensive patients with type 2 diabeteseffect of metaformine and rosiglitazone.Hormones,7(1):70-76.
56. Yener,S.,Dermir,T. and Akinci,B (2007): Transforming growth factor –beta -1 levels in women with prior history of gestational diabetes.Diabetes. Res.Clin.Pract.,76:193-198
57. Zhang,W.,Zhao,J.,Wang,J.,Pang,X.,Zhuang,X.,Zhu,XandQu,W (2010): Hypoglycemic effects of aqueous extract of sea buckthorn seed residue in streptozotocin-induced diabetic rats .phytother res.,24:228-232
58. Zhang,Z.,Jiang,J.,Yu,p.,Zeng,X.,Larrick.J.W and Wang,Y (2009):Hypoglycemic and beta cell protective effect of andrographolide analogue for diabetes treatment .J.Transl.Med.,16:7-62
59. Zhongmin,M.,Maffei,M.,Ahern,B and Scott.p (1996):Radio-immunoassay of leptin in human plasma.Clin.Chem.,42:942-946