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Glycemic Control and Therapeutic Effect of *Nigella sativa* and *Curcuma longa* on Rats with Streptozotocin-induced Diabetic Hepatopathy

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Abstract: This study investigated the possible antidiabetic role and therapeutic crucial action of two medicinal plants namely *Curcuma longa* L. (Zingiberaceae) rhizome and *Nigella sativa* L. (Ranunculaceae) seeds compared to the currently available antidiabetic drug gliclazide (diamicron) against diabetic complication induced liver injury in rats. Experimental diabetes was induced by a single-dose (40 mg kg^{-1}, intraperitoneally, i.p.) streptozotocin (STZ)-injection and the two studied plants were administered orally (300 mg kg^{-1} b.wt. either each alone or in their synergistic combination) for 30 days commenced 2 weeks after induction of diabetes. The following parameters were measured: blood glucose (marker of hyperglycemia), blood fructosamine, hemoglobin (Hb) and albumin (indices of diabetic protein glycation), hepatic glycolytic enzymes, hexokinase (HK), pyruvate kinase (PK) and lactate dehdrogenase (LDH) as well as hepatic gluconeogenic enzyme, phophoenolpyruvate carboxykinase (PEPCK) (to assess the mechanism (s) of hypoglycemic action of the used plants), hepatic oxidative stress markers, Nitric Oxide (NO) and malondialdehyde (MDA, marker of lipid peroxidation), hepatic antioxidant markers including superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and reduced glutathione (GSH). Blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also measured as markers of liver function. The results revealed that induction of diabetes induces metabolic disorder and oxidative hepatopathy indicated by the deviation in the above markers in both blood and livers of diabetic rats. Oral administration of either *C. longa* rhizome or *N. sativa* seeds or their synergistic combination successfully modulated the diabetic increase in blood glucose and fructosamine to their normal levels as well as the consequence diabetic decrease in the Hb and albumin levels, indicating their potential antidiabetic and antiglycating abilities. The plants also effectively have beneficial action in up-regulating of hepatic glycolytic enzymes and down regulating the gluconeogenic enzyme which have the major role in diabetic hyperglycemia and this may demonstrate the mechanisms of glycemic control of these plants. Furthermore, ingestion of the current plants effectively modulated hepatic oxidative tissue damage indicated by amelioration of the deterioration occurred in oxidative stress and antioxidants markers in hepatic of diabetic animals and ensured by normalization of liver function blood enzymes activities, confirming their potential antioxidant activity. Supplementation of diabetic animals with gliclazide modulated diabetic induced alteration in most of the above studied markers. These results suggest that either *C. longa* rhizome or *N. sativa* seeds or their synergistic combination have multi-beneficial actions in controlling diabetes and consequence complication induced in liver and may candidate as natural antidiabetic drugs.

Key words: Antidiabetic, plants, glycation, glycolytic enzymes, oxidative stress

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INTRODUCTION

Diabetes mellitus is a serious, complex chronic condition which is a major source of ill health all over the world. This metabolic disorder affects approximately 4% of the population worldwide and is expected to increase by 5.4% in 2025 (Kim *et al*., 2006). Diabetes mellitus is a disease due to abnormality of carbohydrate metabolism and characterized by absolute (type I) or relative (type II) deficiencies in insulin secretion or receptor insensitivity to endogenous insulin, resulting in hyperglycemia (Alberti and Zimmet, 1998).

Liver is the key organ in the maintenance of systemic glucose homeostasis in mammals. It regulate blood glucose level within a very narrow range under various nutritional conditions, directing excess glucose immediately following a meal to various storage forms and providing glucose to the systemic circulation to fuel the function of many organs and tissues in the fasting state (Klover and Moony, 2004). The fundamental mechanism underlying hyperglycemia in diabetes involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues (Klover and Mooney, 2004).

Hyperglycemia resulting from unregulated glucose level is widely recognized as the causal link between diabetes and diabetic complications (Brownlee, 2001). It was found that hyperglycemia cause tissue damage by mechanisms involving repeated changes in cellular metabolism (Robertson, 2004). One of the key metabolic pathways as being major contributors to hyperglycemia induced cell damage, is the nonenzymatic reaction between excess glucose and several proteins (as hemoglobin and albumin) to form Advanced Glycosylated End (AGE) product (Sheela and Augusti, 1992; Lapolla *et al*., 2005). Production of AGE interferes with cell integrity by modifying protein function or by inducing receptors mediated production of Reactive Oxygen Species (ROS) (Thornalley, 2002).

Hyperglycemia-evoked oxidative stress plays a crucial role in the development of diabetic complications, including nephropathy, neuropathy, retinopathy and hepatopathy, which is considered to result from augmented reactive oxygen species generation (Brownlee, 2001; Hamden *et al*., 2008) which probably results both from an excessive generation of reactive oxygen species and decreased antioxidant defenses (Jin *et al*., 2008). Studies have shown that hepatobiliary disorders, such as the inflammation, necrosis or fibrosis of non-alcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma, hepatitis C, acute liver failure and cholelithiasis can follow diabetes (Latry *et al*., 1987; Tolman *et al*., 2004). Cirrhosis is one of the leading causes of death in diabetics. The incidence of hepatocellular carcinoma is increased in patients with diabetes with hepatocyte lipid accumulation and oxidative stress with the formation of reactive oxygen species. Oxidative stress leads to both DNA damage and cell death (Tolman *et al*., 2004).

The current treatment although provide good glycemic control but do a little in preventing complications. Besides, these drugs are associated with side effects (Berger, 1985). Moreover, providing modern medical health care across the world (especially in developing countries such as India and Egypt) is still a far-reaching goal due to economic constraints. Thus, it is necessary that we continue to look for new and if possible more efficacious drugs and the vast reserves of phytotherapy may be an ideal target. In any form of management of diabetes with insulin or drug, diet is a common factor. With respect to diet, plants and foods of medicinal value have proved to be very useful and are in wide usage as they combine two basic central factors: food and medication (Grower *et al*., 2002) and might represent new therapeutic targets for diabetes treatment.

Curcuma longa L. (Family, Zingiberaceae) has been used for hundreds of years as a flavor, color and preservative. Commercially, it is traded as a dye, spice and source of industrial starch (Cousins *et al*., 2007). The active ingredient, Curcumin (deferuloymethane), obtained from its rhizome has been used in indigenous herbal medicine for the treatment of inflammatory and liver disorders. Curcumin has potent antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic properties

(Araujo and Leon, 2001; Chainani-Wu, 2003). The protective effects of curcumin against chemically-induced hepatotoxicity are well documented and have been attributed to its intrinsic antioxidant properties (Iqbal *et al*., 2003; Rukkumani *et al*., 2004). Efficacy of *Curcuma longa* to reduce blood sugar in diabetic rats was also reported by Arun and Nalini (2002).

Nigella sativa L., commonly known as black seed, belongs to the botanical family Ranunculaceae. It has been in use in many Middle Eastern countries as a natural remedy for over 2000 years (Swamy and Tan, 2000). Black seed components display a remarkable array of biochemical, immunological and pharmacological actions, including bronchodilatory (Schleicher and Saleh, 1998), anti-inflammatory (Mansour and Tornhamre, 2004), immunopotentiating (El-Gazzar *et al*., 2006) and hypoglycemic (Al-Rowais, 2002) effects. *N. sativa* oil have been used for treatment of experimentally induced diabetes in animals based on its combined hypoglycemic and immunopotentiating effects that help in ameliorating the impaired immunity and infections associated with diabetes (Deresinski, 1995).

However, the mechanism underlying the hypoglycemic effect of either *Curcuma longa* rhizome or *N. sativa* seeds is unclear and their hepatoprotective effects against diabetic hepatopathy have never been investigated in diabetic animals. The present study was designed to investigate the possible hypoglycemic effect and mechanism(s) of the of the powder of either *C. longa* rhizome or *N. sativa* seeds or their synergistic combination compared to the currently available antidiabetic drug, gliclazide (diamicron) especially with respect to hepatic glycolysis and gluconeogenesis. The current study also was extended to investigate the possible therapeutic beneficial effects of the used drugs against liver oxidative damage associated with diabetic complications in diabetic rats.

MATERIALS AND METHODS

This study was conducted during winter.

Chemicals

All chemicals used were of high analytical grade, product of Sigma and Merck companies. Kits used for the quantitative determination of different parameters were purchased from Biogamma, Stanbio, West Germany.

Plants

Powders of *C. longa* rhizome and *N. sativa* seeds were purchased from the local market and suspended in 1% Tween 80 before administration.

Animals

One hundred male albino rats (150-200 g) were obtained from animal house of National Research Center, Dokki, Giza, Egypt. Rats were fed a standard diet and free access to tap water. They were kept for 2 weeks to acclimatize to the environmental conditions.

Experimental Design

The rats were divided into two classes: Class 1: consists of 5 normal healthy groups (groups 1-5, each of 10 rats).

- **Group 1:** Normal control rats (not received any medication)
- **Group 2:** *C. longa*-treated group
- **Group 3:** *N. sativa*-treated group
- **Group 4:** Synergistic group, treated with both *C. longa* and *N. sativa*
- **Group 5:** Gliclazide-treated group

Curcuma longa and *N. sativa* were given orally in a dose 300 mg kg^{-1} b.wt. day^{-1} either each alone or in their synergistic combination for 30 days (Hussain, 2002; Kaleem *et al*., 2006). Gliclazide was given orally (10 mg kg^{-1} day^{-1}) (Dachicourt *et al*., 1998) for 30 days.

Class 2, consists of 5 diabetic groups (groups 6-10, each of ten rats), diabetes was induced by streptozotocin (STZ), each rat was injected intraperitoneally with a single dose of streptozotocin (40 mg kg^{-1} b.wt.) dissolved in 0.01 M citrate buffer immediately before use (Milani *et al*., 2005). After injection, they had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock (Bhandari *et al*., 2005). After 2 weeks hyperglycemic rats (460-500 mg dL^{-1}) were used for the experiment.

- **Group 6:** Diabetic control group
- **Group 7:** Diabetic animals treated with *C. longa*
- **Group 8:** Diabetic animals treated with *N. sativa*
- **Group 9:** Diabetic animals treated with synergistic combination of both *C. longa* and *N. sativa*
- **Group 10:** Diabetic animals treated with gliclazide

All the drugs were given as the same manner of healthy groups commenced 2 weeks after induction of diabetes. After 30 days of drugs treatment, the animals were fasted overnight (12-14 h), the blood samples were collected from each animal in all groups into sterilized tubes for serum separation and into tubes containing heparin for hemoglobin determination. Serum was separated by centrifugation at 3000 x g for 10 min and used for biochemical serum analysis. After blood collection, rats of each group were sacrificed under ether anesthesia and the liver samples were collected, minced and homogenized in ice cold bidistilled water to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 min at 10000 x g at 4°C and the supernatants were used for different biochemical tissue analysis.

Biochemical Analysis

All the following biochemical parameters were measured spectrophotometrically.

Blood Analysis

Hb was estimated in the whole heparinized blood by cyanmethemoglobin method (Drabkin and Austin,1932).

Serum Analysis

Fasting blood glucose was measured according to method adopted previously by Miwa *et al*. (1972) using a glucose kit (enzymatic method) (Wako). Fructosamine (glycated serum protein) was determined using reagents, calibrators and controls from Sigma Diagnostics (St. Louis, MO) and application parameters for the Cobas Mira automated chemistry analyzer. The assay is a modification of the original method of Parlin *et al*. (1997) where fructosamine reduces Nitro-Blue Tetrazolium (NBT) under alkaline conditions and forms a purple-colored formazan with an absorption maximum at 530 nm. Albumin was determined using the method of Doumas *et al*. (1971). ALT and AST activities were determined according to the method described by Bergmeyer *et al*. (1986).

Hepatic Tissue Analysis

Enzyme Determination

HK was measured by the method of Gumaa and McLean (1972). The activity of the enzyme was determined as the rate of the reaction mixture contained the following in final concentration of: 0.1 M Tris-HCl (pH 7.4), 8 mM $MgCl_2$ (pH 7.0), 0.4 mM $NADP^+$, 8/2 mM of ATP/Mg_2^+ (pH 7.2),

0.5 mM glucose and one unit of purified glucose-6-phosphate dehydrogenase. The reaction was initiated by addition of 0.1 mL of tissue extract. The reduction of NADP at 340 nm was followed for 5 min as a measure of enzyme activity. PK activity was measured as the rate of decrease in extinction at 340 nm due to the oxidation of NADH by coupling the system with excess of LDH (Bucher and Pfleiderer, 1955). LDH activity was evaluated in a reaction mixture containing tris buffer (50 mM, pH 7.5), sodium pyruvate (0.6 mM) and NADH (0.18 mM). The rate of NADH consumption is determined at 340 nm and is directly proportional to the LDH activity (Bergmeyer, 1975). PEPCK was assayed according to Suarez *et al.* (1986). The method is based on the oxaloacetate formed from phosphoenol pyruvate was determined by measuring the oxidation of NADH in the presence of malate dehydrogenase at 340 nm. SOD activity was determined by monitoring the decrease in absorbance at 340 nm using the method of Paoletti *et al.* (1986). The activity was expressed in terms of % inhibition of NADH. CAT was determined by monitoring the decomposition of hydrogen peroxide as described by Aebi (1984). GR activity was measured by the modified method of Erden and Bor (1984). The reaction mixture contained the following in the final concentration: 4.1 mM tris-HCl, pH 7.5, 15 mM $MgCl_2$, 5.7 mM EDTA, 60 mM KCl, 2.6 IU GSSG and 0.2 mM of NADPH in final reaction volume of 1 mL. The reaction was started by the addition of tissue extract containing approximately 100 µg of protein. The decrease in absorbance was monitored at 340 nm. Tissue protein content determined by the method of Bradford (1976) using bovine serum albumin as standard.

Metabolite Determination

Nitrite concentration (an indirect measurement of NO synthesis) was assayed using Griess reagent (sulfanilamide and N-1-naphthyl ethylenediamine dihydrochloride) in acidic medium (Moshage *et al.*, 1995). Lipid peroxidation was determined by measuring the formed MDA (an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method (Buege and Aust, 1978). This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and MDA, the product of lipid peroxidation was measured at 532 nm. MDA concentration was calculated using extinction coefficient value (ε) of MDA-thiobarbituric acid complex (1.56×10^5/M/cm). The reduced glutathione (GSH) was determined using the method of Bentler *et al.* (1963) based on its reaction with 5,5'-dithiobis (2-nitrobenzoic acid) to yield the yellow chromophore, 5-thio-2-nitrobenzoic acid at 412 nm.

Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as Mean±SD. The significant differences among values were analyzed using analysis of variance (one-way ANOVA) coupled with post-hoc (LSD). Results were considered significant at $p<0.05$.

RESULTS

Table 1 reveals that injection of rats with STZ induced hyperglycemia indicated by significant increase in diabetic marker, fasted blood glucose level in these animals (G 6) as compared to the control ones (Group 1-5). Table 1 also shows an increase in blood fructosamine level with concomitant decrease in Hb and albumin levels versus control animals. Oral administration of either *C. longa* rhizome or *N. sativa* seeds or their synergistic combination (Group 7-9) down-regulated the blood glucose and fructosamine within their normal levels. From the Table 1, it can be observed that the two tested plants each alone or their combination also normalized the decrease in Hb and albumin levels in response to diabetes. Treatment of diabetic animals with the currently available drug, gliclazide

Table 1: Levels of blood glucose, fructosamine, Hb and albumin in normal groups and different diabetic groups

	Normal groups					Diabetic groups				
	G1	G2	G3	G4	G5					
	Normal	*C. longa*	*N. sativa*	Synergestic	Drug	G6	G7	G8	G9	G10
Parameters	-control	(C)	(N)	(S)	(D)	diabetic	Dia+ C	Dia+ N	Dia+ S	Dia+ D
Glucose	81.60±2.2	82.30±2.5	89.0±2	84.6±4	83.3±3.2	483.30±22[a]	90.3±6	91.5±5	83.30±1	00.7±3.7[bc]
Fructosamine	208.30±17	218.00±11.5	227.3±11	220.6±5	220.0±17.6	637.30±22[a]	222.6±15	218.3±8.5	214.30±5	236.0±10[bc]
Hemoglobin	15.30±0.6	16.00±0.7	15.8±1.0	15.8±1.0	16.8±0.6	7.86±0.9[a]	15.5±1	15.5±0.8	15.83±0.8	15.6±1
Albumin	4.40±0.3	4.56±0.05	4.9±0.1	4.7±0.3	4.8±0.15	2.60±0.1[a]	4.7±0.1	4.7±0.4	4.70±0.52	4.8±0.2

Data are expressed as Mean±SD of 6 rats in each group. Glucose is expressed in mg dL^{-1}, fructosamine is expressed in µmol L^{-1}, Hb and albumin are expressed in g dL^{-1}, [a]p<0.0001, [b]p<0.05 when compared with normal group. [c]p<0.0001 when compared with diabetic untreated group

Table 2: Activities of some glycolytic and gluconeogenic enzymes in different experimental normal and diabetic groups

	Normal groups					Diabetic groups				
	G1	G2	G3	G4	G5					
	Normal	*C. longa*	*N. sativa*	Synergestic	Drug	G6	G7	G8	G9	G10
Parameters	-control	(C)	(N)	(S)	(D)	diabetic	Dia+ C	Dia+ N	Dia+ S	Dia+ D
HK	103.00±7	104.60±2.5	100.5±7	101.00±5.9	102.60±5	19.00±2.6[a]	93.30±7[ad]	95.30±5[ad]	90.3±5[ad]	85.5±3.6[bd]
PK	55.60±3.4	54.80±4.1	52.0±2.1	56.20±3.9	52.70±3.6	15.60±1.52[a]	52.66±2.5	59.40±3.2	55.3±2.5	53.5±1.7
LDH	35.60±3.7	36.30±2.5	34.3±1.5	36.60±2.5	35.10±1.2	8.10±0.98[a]	32.00±4.3	38.30±4	32.0±1	33.6±2
PEPCK	7.73±1.045	8.02±0.78	7.4±0.9	8.03±0.95	8.06±0. 6	13.30±0.65[a]	7.90±0.4	7.80±1	7.5±0.8	8.3±0.6

Data are expressed as Mean±SD of 6 rats in each group. Hk, Pk and LDH are expressed in nmol/min/ mg protein, PEPCK is expressed in µmol/min/mg protein. [a]p<0.0001, [b]p<0.01, [c]p<0.05 when compared with normal group. [d]p<0.0001when compared with diabetic untreated group

Table 3: Levels of oxidative stress and antioxidant markers in normal and diabetic group

	Normal groups					Diabetic groups				
	G1	G2	G3	G4	G5					
	Normal	*C. longa*	*N. sativa*	Synergestic	Drug	G6	G7	G8	G9	G10
Parameters	-control	(C)	(N)	(S)	(D)	diabetic	Dia+ C	Dia+ N	Dia+ S	Dia+ D
MDA	17.20±1.9	19.10±1.85	17.60±1.6	17.7±1.36	17.60±1.6	118.26±2.8a	17.26±1.9	17.28±2.5	17.28±2.5	17.6±3.00
NO	43.03±2.2	42.50±2.7	42.50±1.65	43.3±2.17	44.30±4.3	74.70±2.85a	43.6±2.68	44.38±2.12	45.40±3.22	44.7±1.60
GSH	2.60±0.121	2.77±0.02	2.60±0.03	2.65±0.12	2.73±0.05	1.87±0.06a	2.7±0.07	2.65±0.07	2.68±0.045	2.7±0.07
SOD	7.36±0.35	7.33±0.40	7.40±0.26	7.16±0.25	7.03±0.40	1.33±0.15a	7.0±0.30	7.41±0.125	7.30±0.22	6.9±0.13
CAT	15.70±0.2	15.26±0.2	14.90±0.40	15.76±0.5	14.90±0.4	4.70±0.10a	14.93±0.6	14.90±0.8	15.30±1.00	16.1±0.75
GR	12.00±0.75	11.10±0.8	12.00±1.8	12.90±1.34	12.30±0.77	2.93±0.11a	11.75±0.68	11.73±0.64	12.60±1.3	12.1±1.20

Data are expressed as Mean±SD of 6 rats in each group. MDA is expressed in nmol g^{-1} tissue, NO and GSH are expressed in µmol g^{-1} tissue, SOD and GR are expressed in nmol/min/mg protein, CAT is expressed in µmol/min/mg protein. [a]p<0.0001 when compared with normal group

Table 4: Activities of serum liver function enzymes in different experimental normal and diabetic groups

	Normal groups					Diabetic groups				
	G1	G2	G3	G4	G5					
	Normal	*C. longa*	*N. sativa*	Synergestic	Drug	G6	G7	G8	G9	G10
Parameters	-control	(C)	(N)	(S)	(D)	diabetic	Dia+ C	Dia+ N	Dia+ S	Dia+ D
AST	15.12±2.6	17.44±1.76	18.0±4.33	15.7±1.7	40.1±3.5[a]	110±6.9[a]	19.7±2.6	18.0±2.6	17.4±3	71±3[ab]
ALT	20.16±2	16.10±1.30	17.8±3.9	16.8±3.6	42.7±6[a]	103±7[a]	22.8±2	23.6±2	18.8±3.4	67±5[ab]

Data are expressed as Mean±SD of 6 rats in each group. AST and ALT are expressed in U L^{-1}, [a]p<0.0001 when compared with normal group [b]p<0.001 when compared to diabetic untreated group

(G 10) markedly down regulated blood glucose level, improved the alteration in fructosamine level in relation to diabetic group and ameliorated Hb and albumin within their normal levels.

STZ induced diabetic rats showed marked depletion in hepatic glycolytic enzymes (HK, PK and LDH) accompanied with elevation in the gluconeogenic one (PEPCK). Treatment of diabetic rats with the tested plants either alone or their synergistic combination or gliclazide, significantly normalized the deviation in the most of these enzymes altered under the effect of diabetes (Table 2).

Diabetic rats showed marked increase in hepatic MDA (index of lipid peroxidation) and NO levels parallel with decrease in hepatic SOD, CAT, GR (enzymatic antioxidants) and GSH (non-enzymatic antioxidant) (Table 3). Ingestion of the plants under investigation either alone or their synergistic combination effectively normalized the deterioration occurred in these markers in hepatic of diabetic animals. Supplementation of diabetic animals with gliclazide was found equaled to the current tested plants in modulating diabetic induced deviation in these indices.

Table 4 shows the activities of serum ALT and AST as two markers evaluate liver function in normal and diabetic animal in different experiment groups. From the Table 4, it can be noticed that

induction of diabetes developed significant liver damage as observed from elevated levels of hepatospecific enzymes, serum ALT and AST. Administration of the two candidate plants each alone or in combination successively down-modulate the increase in these serum enzymes within their normal activities, however treatment of diabetic animals with the currently antidiabetic drug, gliclazide improved the alteration in these enzymes.

No significant changes were seen in the studied parameters on treating the normal rats with the used plants either alone or their synergistic combination (Group 2, 3 and 4) compared to normal untreated group (G1), however, treatment of normal animals with gliclazide significant increase in liver function enzymes, serum ALT and AST was observed only.

DISCUSSION

Little scientific evidence exists to support the numerous herbs used to improve diabetes-related metabolic disorders. It is known that considerable medical resources have been invested on the prevention and control of the diabetes-related complications.

The present study has demonstrated that injection of rats with STZ resulted in a significant elevation in blood glucose in diabetic control group as compared with normal animals indicating establishment of diabetic state. Oral administration of diabetic animals with either *C. longa* rhizome or *N. sativa* seeds or their synergistic combination markedly down regulated the blood glucose level. The two tested plants have better hypoglycemic effect than with the currently available antidiabetic drug, gliclazide as they regulated blood glucose of diabetic animals within the level of normal animals, indicating their potential hypoglycemic action. This is consistent with the finding of Kumar *et al*. (2005) reported that feeding *C. longa* to the diabetic rat improved fasting blood glucose and attributed its hypoglycemic effect to the high amount of fiber which facilitate a slower absorption of glucose in the gastrointestinal tract. Also Fararh *et al*. (2004) demonstrated that oral administration of oil extracted from *N. sativa* seeds produced a significant decrease in blood glucose levels in STZ-induced diabetic hamsters.

In line with earlier studies, the current investigation also revealed significant elevation in serum fructosamine level with concomitant decrease in Hb and albumin levels in diabetic animals in relation to normal ones (Montilla *et al*., 2004; Pari and Satheesh, 2004). Serum fructosamine, is a glycated protein which has the crucial role in the progression of many pathological conditions (Sabater *et al*., 1991; Misciagna *et al*., 2004). It results from spontaneous nonenzymatic condensation of excess glucose present in blood and a number of proteins including Hb and albumin due to uncontrolled or poorly controlled diabetes, therefore the total Hb and albumin levels are decreased (Sheela and Augusti, 1992; Bourdon *et al*., 1999; Lapolla *et al*., 2005; Sudnikovich *et al*., 2007). The amount of increase in fructosamine, was found directly proportional to the fasting blood glucose level (Jackson *et al*., 1979). Albumin is the most abundant plasma protein and is a powerful extracellular antioxidant (Bourdon *et al*., 1999). It contains 17 disulphide bridges and has a single remaining cysteine residue which is responsible for the capacity of albumin to react with and neutralize peroxyl radicals (Young and Woodside, 2001). Decreased in albumin due to its glycation during diabetes may consider one of the important factors responsible for oxidative stress related to diabetes (Jin *et al*., 2008).

Ingestion of either *C. longa* rhizome or *N. sativa* seeds or in combination effectively normalized the deterioration in fructosamine, Hb and albumin levels which may attributed to their potential glycemic control together with they may have an important role in preventing protein glycation. Present results as documented by previous investigation has shown that curcumin (active principle of *Curcuma longa* rhizome) inhibits advanced glycation end products in STZ diabetes in rats (Taniguchi *et al*., 1996). Also, it was found that treatment with *N. sativa* oil significantly reduced total glycated hemoglobin, compared with the untreated diabetic animals which reflects the adequate and effective action of *N. sativa* in long-term reduction of diabetic hyperglycemia (Fararh *et al*., 2004).

Administration of diabetic animals with gliclazide was also found to have an inhibitory effect on the glucose-induced glycation during diabetic state indicated by improvement of fructosamine level and normalization of Hb and albumin levels. This is as in earlier study which provides the first evidence of the antiglycation effect of gliclazide on *in vitro* AGE formation from glucose and methylglyoxal (Li *et al*., 2008).

The present study was extended to assess the biochemical mechanism(s) of the action of glycemic control of the plants under investigation compared to the antidiabetic drug, gliclazide. Liver glycolytic and gluconeogenic enzymes are the key enzymes responsible for maintaining the homeostasis of the blood glucose (Maiti *et al*., 2004). A significant reduction of glycolytic enzymes HK, PK (the rate limiting enzymes) and LDH in livers of diabetic animals accompanied by a marked increase in the gluconeogenic enzyme, PEPCK (a key rate-controlling enzyme in the pathway of gluconeogenesis) were documented in the current study. These results are consistent with earlier reports which stated that diabetes is developed due to obstruction of glucose utilization by the tissues through glycolysis and its over-production through excessive hepatic gluconeogenesis (Klover and Mooney, 2004) and this may attribute to the relative deficiency of insulin which is responsible for increasing the expression of the glycolytic enzymes HK and PK and regulating gluconeogenic enzymes in the liver (Sun *et al*., 2002). Administration of the studied plants either each alone or their synergistic combination normalized the alteration in most of these enzymes in hepatic of diabetic rats which enlightened their possible way of antidiabetogenic activity. This is the first study which demonstrated the mechanism of glycemic control of the two tested plants. In this context a number of other plants have been observed to have similar patterns of hypoglycemic effects (Sudnikovich *et al*., 2007). This effect was also seen with gliclazide supplementation to diabetic rats which may consider another mechanism to its glycemic control in addition to its previously reported role in triggering insulin release from the pancreatic beta cell (Rendell, 2004).

Oxidative stress is known to play a pivotal role in development of diabetes. An imbalance of oxidant/antioxidant in favor of oxidants contributes to the pathogenesis of diabetes (Sudnikovich *et al*., 2007; Jin *et al*., 2008). Data presented in this investigation indicate that induction of diabetes results in augmentation of oxidative stress in livers of diabetic rats accompanied by impaired antioxidative defense, as indicated by significant elevation in the levels of liver oxidative stress markers, NO and MDA (index of lipid peroxidation) with parallel depletion of free-radical scavenging antioxidants, SOD, catalase, GR and GSH. These results may be due to hyperglycemia induced auto-oxidation of lipids and glycation of protein/glucose, result in formation of Reactive Oxygen Species (ROS) and Nitrogen Species (RNS) which have the major role in diabetic complications (Szkudelski, 2001; Sudnikovich *et al*., 2007). Production of NO exert various influences on the pathogenesis of tissues (Mohamed *et al*., 2001). Its pathogenesis is enhanced by reacting with superoxide radical to give powerful secondary toxic oxidizing species, such as peroxynitrite ($ONOO^-$) which is capable of oxidizing cellular structure and causes lipid peroxidation (Mohamed *et al*., 2001) which has often been considered the proximal cause of cell death. Lipid peroxidation can damage protein, lipids, carbohydrates and nucleic acids. Plasma membranes are the critical targets of lipid peroxides (Freeman and Crap, 1982). It also has been found that lipid peroxidation is one of the risk factor of protein glycation (Selvaraj *et al*., 2006). On the other hand, decline in the activities of free-radical scavenging enzymes, SOD and catalase, may be due to inactivation caused by excess reactive oxygen species production. This damages the first line of enzymatic defense against superoxide anion and hydrogen peroxide. The significant depletion of GR and GSH indicating damage to the second line of antioxidant defense. This probably further exacerbates oxidative damage by adversely affecting critical GSH-related processes such as free-radical scavenging, detoxification of electrophilic compounds, modulation of cellular redox status and thiol-disulphide status of proteins and regulation of cell signaling and repair pathways. Reduced antioxidant levels as a result of increased ROS production in experimental diabetes has been previously reported by Lee *et al*. (2005) and Liu *et al*. (2008).

Supplementation of the plants under investigation either alone or in combination to diabetic animals, effectively normalized the deviation in most of these oxidative stress markers in their livers, implying their antioxidant abilities. The possible mechanism which may explain the beneficial effect of these drugs against diabetic hepatopathy is that the used drugs by their antioxidant potential action have excellent effects against oxidative damage and reactive species induced in liver by diabetes. *C. longa* powder by itself and its major bioactive component curcumin, is known to protect against oxidative stress (Zafir and Banu, 2007; Bayrak *et al*., 2008) by typical radical-trapping ability as a chain-breaking antioxidant (Sreejayan Rao, 1994). It also contains other antioxidant principles besides curcumin, such as demethoxycurcumin, bisdemothoxycurcumin and a number of polypeptides with antioxidant activity, such as turmerin (Ramirez-Tortosa *et al*., 1999). Dietary turmeric is known to lower lipid peroxidation by enhancing the activities of antioxidant enzymes (Pulla Reddy and Lokesh, 1994). Also, the antioxidant effects of *N. sativa* have been examined using hepatopathy in different experimental pathological conditions. The crude oil and its fractions (neutral lipids, glycolipids and phospholipids) showed potent *in vitro* radical scavenging activity (Ramadan *et al*., 2003). In addition, previous published data provide clear evidence that the volatile oil, thymoquinone (TQ) possess reproducible antioxidant effect through enhancing the oxidant scavenger system, inhibiting membrane lipid peroxidation and nitrite production, a parameter for NO synthesis (El-Mahmoudy *et al*., 2002; Mahmoud *et al*., 2002; Ali, 2004). Also, similar effect was obtained with supplementation of gliclazide suggesting that it may protect against the oxidative stress-related chronic diabetes complications (Sliwinska *et al*., 2008).

In parallel with earlier report, the present study revealed that in diabetic rats the activities of serum AST and ALT (markers of liver tissue damage) were significantly increased relative to their normal levels (Hickman *et al*., 2008). Supporting present finding, it has been found that hyperglycemia (25 days) resulted in hepatolysis reflected by increased blood plasma aminotransferase as one of the consequences of diabetic complication (Mansour *et al*., 2002). The increment of such markers may be due to the leakage of these enzymes from the liver cytosol into the blood stream. Treatment of the diabetic rats with the tested plants either each alone or in combination caused normalization in the activities of these serum enzymes, indicating their potential ability in inhibiting liver damage induced by diabetic status. However, treatment with gliclazide slightly improve the diabetic increase in these liver enzymes which is coincided with earlier published data revealed that patients receiving gliclazide had relatively abnormal liver functions (Belcher and Schernthaner, 2005).

Evaluation of the adverse effects of the natural products, accepted as remedies, is important in implementing safety measures for public health. The present study proved that the used antidiabetic therapeutic dose of either *C. longa* rhizome or *N. sativa* seeds (300 mg kg^{-1} b.wt./day for 30 days) has no adverse effects which is indicated by the normal levels of all tested parameters in rat livers administered these plants each alone or their synergistic combination(Gs2, 3 and 4) in comparison to normal untreated rats. However, significant increase in liver function enzymes, ALT and AST was found on treating normal with gliclazide (G5). This is in coped with some researchers reported that this drug may induce in some cases acute hepatitis indicated by marked portal inflammation with lymphocytes, monocytes and eosinophils, associated with lobular inflammation (Dourakis *et al*., 2000).

In conclusion, the present study demonstrated that both *C. longa* rhizome and *N. sativa* seeds, have beneficial glycemic control as well as they have principal role in preventing the liver damage caused by hyperglycemia. Hence, with their antiglycating and antioxidant features, treatment with the two plants either each alone or in combination, at the safely effective therapeutic dose used in the current study, can be effective in the recovery of liver tissue from the damage induced by diabetes and may candidate as natural antidiabetic drugs.

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