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Attenuation of Some Metabolic Deteriorations Induced by Diabetes Mellitus Using Carnosine

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Abstract: The protective ability of carnosine against some metabolic disorders and oxidative stress in Strepotzotocin (STZ) diabetic-induced model was studied. Diabetic rats showed significant increase in serum glucose and cortisol levels indicating disturbance of carbohydrate metabolism, increased triglycerides, total cholesterol, LDL-cholesterol as well as iron level indicating abnormal lipid metabolism and iron overload. Marked increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and sorbitol dehydrogenase (SD) were also demonstrated implying impairment of liver function. Concomitantly, the results revealed an impairment of antioxidant status of diabetic animals as evidenced by significant decrease in vitamin E and HDL-C levels. Administration of either two doses of carnosine (10 mg/100 g b.w. or 20 mg/100 g b.w.) two weeks before and after diabetic induction, was effective in ameliorating serum glucose level of diabetic animals and improving the deterioration in the studied parameters. The best results were obtained with the higher dose. No significant changes were noted in serum bilirubin level among the different studied groups. These data suggest that carnosine is a potential multi-protective agent for diabetic complications prevention or therapy.

Key words: Carnosine, diabetes, serum, glucose, cortisol, lipid profiles, iron, liver functions

INTRODUCTION

Type I diabetes mellitus (DM) is an endocrine disorder, which characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with high glucose level. It was found that diabetic complications such as neuropathy, hepatopathy, nephropathy, aging and atherosclerosis contribute to the severity and mortality of diabetic patients (Rosen *et al*., 2001; Brownlee, 2001; Vincent *et al*., 2002). The clinical characteristics of these complications include hyperglycemia, hypercortisolemia, hyperlipidemia as well as transition metal overload (Amaral *et al*., 2002; Strippoli *et al*., 2003; Goldberg, 2003; Ikeda *et al*., 2006; Radahmadi *et al*., 2006). Thus it is important to control these risk factors in order to improve diabetic complications. In modern medicine, no satisfactory effective therapy is still available to cure DM (Sumana and Suryawashi, 2001). It can be managed by exercise, diet and chemotherapy. However, the pharmaceutical drugs are either too expensive or have undesirable side effects (Berger, 1985). Though insulin therapy is also used for the management of DM but there are several drawbacks like insulin resistance (Piedrola *et al*., 2001) anarexia nervosa, brain atrophy and fatty liver (Yaryura-Tobias *et al*., 2001) after chronic treatment.

Oxidative stress is considered one of the primary causative factors that link diabetes with the pathogenic complications of several tissues (Duckworth, 2001; Anwar and Meki, 2003). This stress results from an imbalance between the production of free radicals (reactive oxygen species, ROS) and the effectiveness of the antioxidant defense systems (Abou-Seif and Youssef, 2001, 2004).

Treatment strategies that focus on using antioxidant drugs to decrease oxidative stress, might represent new therapeutic targets for diabetes treatment.

The antioxidant carnosine (beta-alanyl-L-histidine) naturally occurring in animal tissues was reported for its antioxidant, antiglycating effects as pH-buffer and as heavy metal chelator (Babizhayer *et al*., 1994; Lee *et al*., 1999; Ukeda *et al*., 2002). Further studies indicated that carnosine affect glycemic control in diabetic animals (Nagai *et al*., 2003; Hwang *et al*., 2003). However, it is still unknown if this antioxidant could improve the metabolic complications induced in response to diabetic stress. So the major purpose of this study was to evaluate the influence of oral administration of carnosine on the serum levels of some biochemical markers of metabolic disorders and oxidative stress in streptozotocin induced-diabetic rats.

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MATERIALS AND METHODS

Chemicals: Chemicals were of analar quality products of Meck, Germany and Sigma, USA.

Animals: Forty adult healthy male albino rats with body mass of approximately 200-230 g were used. Rats were purchased from animal house of Faculty of Medicine, Cairo University. The animals were conditioned at room temperature and at natural photoperiods for one week before study. A commercial balanced diet and tap water *ad libitum* were provided.

Induction of experimental diabetes: Thirty rats were injected intraperitoneally with a single dose of streptozotocin (40 mg kg^{-1} body weight) dissolved in 0.01 M citrate buffer immediately before use (Emerick *et al*., 2005; Milani *et al*., 2005). Three days later, blood glucose levels were determined in this groups in whole blood samples collected from the tip of the tail. The rats injected with STZ were considered as diabetic if the fasting blood glucose levels was >200 mg dL^{-1} (DeCarvahlo *et al*., 2005).

Experimental design: The rats were divided into 4 groups (each of 10 rats) after the induction of diabetes.

Group 1: Normal rats, received saline solution by intramuscular injection.

Group 2: Diabetic control.

Group 3: Diabetic rats were injected intramuscular with carnosine 10 mg/100 g body weight daily for four weeks.

Group 4: Diabetic rats were injected intramuscular with carnosine 20 mg/100 g body weight daily for four weeks.

At the end of experimental period (4 weeks) the animals were fasted overnight (12-14 h), the blood samples were collected and serum was separated for biochemical analysis.

Biochemical serum analysis: Serum glucose level was measured by the glucose oxidase method (Goldstein *et al*., 1994). Serum vitamin E was measured using the Emmerie-Engel reaction, which is based on the reduction of ferric ions to ferrous ions by α-tocopherol, forming a red complex with α, α-dipyridyl, α-tocopherol was used as standard (Roe, 1961). Serum cortisol level was estimated using Elisa Kits (Arakawa *et al*., 1979). Serum triglycerides were determined using enzymatic colorimetric kits (Wahelfed, 1974). Both total cholesterol and HDL-C were estimated in serum according to the method described by Stein, (1986). According to Friedewald *et al.* (1972), LDL can be calculated as Follows:

$$LDL = \text{total cholesterol-HDL-TG/5}$$

Serum iron was analyzed by colorimetric method (Weissman and Leggi, 1974). ALT and AST were estimated in serum through measuring oxaloacetate and pyruvate produced, respectively (Bergmeyer and Bernt, 1974). Serum SDH was determined by using fructose as substrate (Bergmeyer, 1974). Finally, bilirubin was determined using the method of Sherolck (1951).

Statistical analysis: Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as the mean±SD the significant differences among values were analyzed using one-way ANOVA coupled with post-hoc (LSD).

RESULTS AND DISCUSSION

Serum biochemical profiles of the studied four groups are shown in Table 1-3. A significant increase in serum glucose, cortisol, triglycerides, total cholesterol, LDL-cholesterol and iron as well as ALT, AST and SDH levels was observed in diabetic animals (G_2) in comparison to non-diabetic ones (G_1). While, a significant decrease in vitamin E and HDL-cholesterol was detected. Normalization of serum glucose level was observed with carnosinc administration using either of the two doses (10 mg/100 g or 20 mg/100 g b.w. i.m) (G_3 and G_4, respectively), however, improvement of the metabolic disorders induced in other parameters in response to DM was proved, the best results were shown with higher dose (20 mg/100 g b.w.). No significant changes was seen in bilirubin level among the experimental different groups.

The change in the above tested markers given as a percent of control is illustrated in Fig. 1-3.

Antioxidant therapy aiming to reduce the extent of free radical-mediated tissue damage would represent a rational approach in preventing the onset and/or progression of free radicals related debilitations (Ravikumar and Anuradha, 1999). It has been emphasized that free radicals and oxidative stress play an important role in the pathogenesis of diabetes and its later complications (Robertson, 2004).

The present study focuses on the corrective effect of carnosine (as antioxidant agent) on some metabolic complications induced by diabetes. The current data revealed a significant increase in serum glucose level

Table 1: Effect of carnosine administration on serum glucose, vitamin E and cortisol levels in different studied groups

Parameters	Control group (G_1)	Diabetic group (G_2)	Carnosine-treated groups (G_3)	Carnosine-treated groups (G_4)
Glucose (mg dL^{-1})	106.00±9.00	482.0±22.65	110.25±6.8	113.41±8.70
LSD	(2)	(1,3,4)	(2)	(2)
Vit. E (mg dL^{-1})	4.24±0.12	1.98±0.41	3.16±0.62	3.60±0.53
LSD	(2,3)	(1,3,4)	(1,2)	(2)
Cortisol (ng mL^{-1})	163.06±11.62	381.03±11.09	229.01±16.2	169.46±12.3
LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)

Data are expressed as mean±SD of 10 rats in each group, ANOVA at $p<0.0001$

Table 2: Effect of carnosine administration on serum lipid profiles and iron levels in different studied groups

Parameters	Control group (G_1)	Diabetic group (G_2)	Carnosine-treated groups (G_3)	Carnosine-treated groups (G_4)
Total cholesterol (mg dL^{-1})	185.66±13.00	480.5±27.54	364.00±8.64	290.00±39.93
LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)
Triglycerides (mg dL^{-1})	66.73±8.26	166.29±11.07	92.51±24.71	62.86±25.25
LSD	(2)	(1,3,4)	(2,4)	(2,3)
LDL (mg dL^{-1})	110.96±8.55	354.26±32.16	301.75±40.8	148.41±17.19
LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)
HDL (mg dL^{-1})	67.88±5.65	36.14±5.62	68.77±2.09	86.12±9.90
LSD	(2,4)	(1,3,4)	(2,4)	(1,2,3)
Iron (µg dL^{-1})	95.34±9.60	120.10±15.60	92.67±10.91	90.85±10.02
LSD	(2)	(1,3,4)	(2)	(2)

Data are expressed as mean±SD of 10 rats in each group, ANOVA at $p<0.0001$

Table 3: Effect of carnosine administration on liver function in serum of different studied groups

Parameters	Control group (G_1)	Diabetic group (G_2)	Carnosine-treated groups (G_3)	Carnosine-treated groups (G_4)
ALT (U L^{-1})	41.17±3.39	110.54±10.51	48.88±4.16	46.93±2.39
LSD	(2)	(1,3,4)	(2)	(2)
AST (U L^{-1})	41.85±1.67	106.10±9.16	58.62±11.02	55.93±7.92
LSD	(2,3,4)	(1,3,4)	(1,2)	(1,2)
SDH (µ mole NADH min^{-1} mL^{-1})	0.03±0.02	0.09±0.024	0.048±0.014	0.046±0.019
LSD	(2)	(1,3,4)	(2)	(2)
Total bilirubin (mg dL^{-1})	1.033±0.38	1.277±0.178	1.199±0.238	1.36±0.164
LSD	(ns)	(ns)	(ns)	(ns)

Data are expressed as mean±SD of 10 rats in each group. ANOVA at $p<0.0001$ NS: Non Significant changes between groups

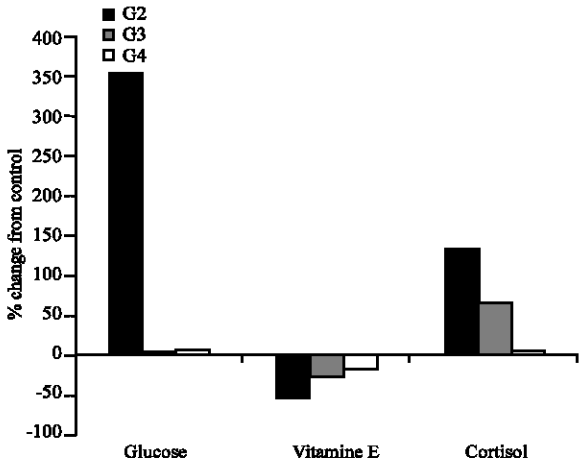

Fig. 1: Glucose, vitamin E and cortisol in serum of rats given as percent of control

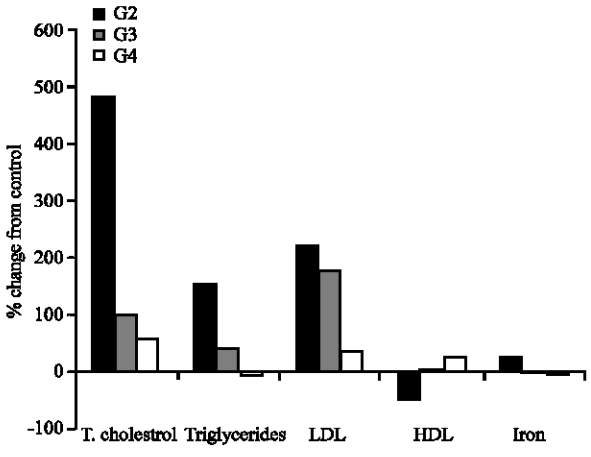

Fig. 2: Lipid profiles and iron in serum of rats given as percent of control

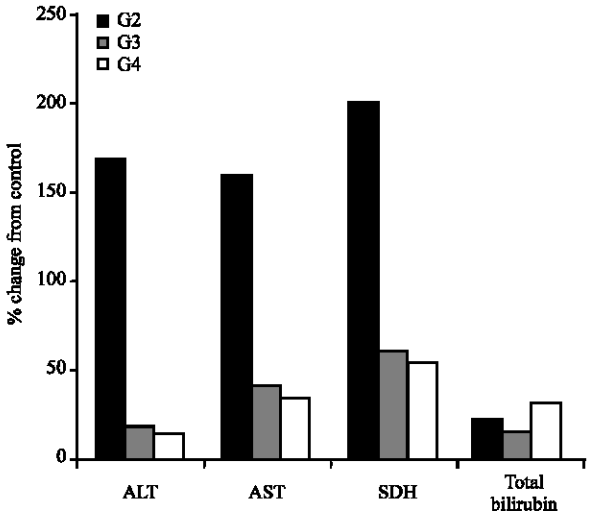

Fig. 3: ALT, AST, SDH and total bilirubin in serum of rats given as percent of control

induced by STZ, indicating establishment of the diabetic state (Hardman and Limbird, 2001). STZ is an antibiotic and anticancer agent which is widely used for inducing type 1 diabetes in a variety of animals (Merzouk *et al*., 2000). It selectively induces degenerative alterations and necrosis of pancreatic β-cells resulting in insulin deficiency (DeCarvalho *et al*., 2005).

The fundamental mechanism underlying hyperglycemia in DM involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose (Latner, 1958). Hyperglycemia resulting from uncontrolled glucose regulation is the causal link between diabetes and diabetic complications (Brownlee, 2001).

It was emphasized that, exposure to high glucose level causes tissue damage by mechanisms involving repeated acute changes in cellular metabolism (Robertson, 2004). One of the key metabolic pathway as being major contributor to hyperglycemia-induced cell damage, is the non-enzymatic reaction of glucose with many proteins (transition metal-catalyzed process ions) resulting in the formation of advanced glycosylated end products (AGEs). AGEs act as oxidants, bind to specific receptors on the cell surfaces, leading to the production of Reactive Oxygen Species (ROS) (Yan *et al*., 1994; Thornalley, 2002).

Treatment of diabetic rats with carnosine at low and high concentrations presented in our study, significantly reduced blood glucose level, indicating its hypoglycemic action. This result is in line with previous studies which revealed that carnosine could affect glycemic control in diabetic animals (Nagai *et al*., 2003; Hwang *et al*., 2003; Lee *et al*., 2005). This could be explained by some authors who indicated that this agent could alleviate diabetic hyperglycemia via insulin resoration capability (Lee *et al*., 2005), it inhibitis neural activities of sympathetic efferent nerves innervating the adrenal gland and liver and facilitates the activity of vagal celiac nerve innervating the pancreas, causing an increase in insulin secretion and a suppression in the glucagon secretion from the pancreas (Yamano *et al*., 2001; Nagai *et al*., 2003). This suggested that it might be one of the endogenous factors controlling blood glucose level (Lee *et al*., 2005).

It was reported that overproduction of ROS and oxidative stress play a role in reducing antioxidant levels in DM (Gul *et al*., 2002). In the present study, STZ treatment caused a significant depletion of serum non-enzymatic antioxidant vitamin E and carnosine supplementation significantly improved its level. The best result was obtained with the larger dose. Reduced antioxidant levels as a result of increased ROS production in experimental diabetes has been previously reported (Giugliano *et al*., 1995; Krha *et al*., 2003; Anwar and Meki, 2003; Lee *et al*., 2005). However, the corrective action of carnosine could be attributed to its previously proved potent antioxidant ability to inactivate ROS and scavenging free radicals (Baran, 2000; Tromblay *et al*., 2000). It also has an important role as antiglycating agent, inhibiting AGEs (mediated ROS production) formation through its transition metals chelating action (Hipkiss, 1998; Price *et al*., 2001).

It has been reported that diabetes complications of clinical states and changes of biochemical parameters like glucose have a crucial role in autoimmune endocrine diseases (Pleho *et al*., 1994). In line with Radahmadi *et al*. (2006), the current work showed that serum cortisol level was higher in diabetic rats compared to normal ones. This confirms some previous studies stating that diabetes is associated with elevated plasma level of glucocorticoids and increased adrenal function (Cameron *et al*., 1987; Roy *et al*., 1990; Bitar, 1998; Radahmadi *et al*., 2006). This hormonal disbalance might influence the control of diabetes and increase the risk of medical complications (Roy *et al*., 1993).

Cortisol (hydrocortisone) is the most important human glucocorticoid steroid hormone involved in glucose metabolism. Normal circulating level of cortisol help sustain basic physiological function, it stimulates (in fasted state) several processes that collectively serve to increase and maintain normal concentration of glucose in blood (Bhagavan, 2001). However, large amount of cortisol released in response to different stressors including diabetes (Radahamdi *et al*., 2006) enable the individual to cope with metabolic demands, it inhibits glucose utilization in peripheral tissues by inhibiting glycolysis, promoting gluconeogenesis and liberation of fatty acids from adipose tissues. This may play the fundamental role of increased glucose level and fat accumulation associated with DM.

Carnosine administration to diabetic rats have shown a regulatory effect on cortisol level. Present results agree with the result that was obtained in some studies that cortisol released into circulation in response to oxidative stress was quickly metabolized after carnosine administration through enhancing cortisone beta-reductase metabolizing enzyme (Nagai *et al*., 1990). These results provide further evidence by Yan and Harding (2005) that carnosine has antisteroid effect.

In addition to abnormal glucose metabolism, DM often involves abnormal lipid metabolism which is considered an additional metabolic disorder, in diabetic complications (Krentz, 2003). In agreement with some authors, the present data revealed that hyperglycemia produced marked increased level of serum triglycerides, total cholesterol, LDL-cholesterol (LDL-C) with concomitant decrease in HDL-C (Tsutsumi *et al*., 1999; Krentz, 2003; Abou-Seif and Youssef, 2004; Saxena *et al*., 2005). This hyperlipidemia associated with DM may be attributed to insulin deficiency (Morel and Chisolm, 1989) and elevated cortisol level, which have an important role in the process of fat accumulation (Hristova and Aloe, 2006). Under normal circumstances, insulin activates lipoprotein lipase which hydrolyzes triglycerides. Insulin deficiency results in failure to activate the enzyme, thereby causing hypertriglyceridemia (Shirwaikar *et al*., 2004). On the other hand, in insulin deficient diabetic subjects, the plasma free fatty acids concentration is elevated as a result of increased free fatty acids outflow from fat depots, where the balance of the free fatty acid estrification, triglyceride lipolysis cycle is displaced in favour of lipolysis (Shirwaikar *et al*., 2004). Also elevated cortisol promotes the liberation of fatty acids from adipose tissue into blood stream by inducing and maintaining the synthesis of the hormone sensitive lipase (HSL), thus increasing free fatty acids level which contributes to cardio-vascular risk (Lundberg, 2005).

The reduction in cardioprotective HDL-C means a reduction of cholesterol efflux from the tissues, the first step in reverse cholesterol transport from the peripheral tissues to the liver. The antioxidant and antiatherogenic activities of HDL-C are reduced when its circulating level is low. LDL-C particles become small and dense which undergo oxidative modification, thus leading to a diabetic complication, (Kalousova *et al*., 2002).

It was found that increased level of transition metal such as iron in response to DM is closely related to LDL-C oxidation (Ikeda *et al*., 2006; Lee *et al*., 2006). This is consistent with the current study which revealed an increase in serum iron in diabetic animals versus control ones. The increment of serum iron level may be due to its mobilization from ferritin in response to hyperglycemia. Unused iron is stored as ferritin molecules to ensure neutralization (Juckett *et al*., 1995) and iron must be in its free state to act as pro-oxidant agent. It is released from ferritin by the action of a reducing agent that converts Fe^{3+} to Fe^{2+}. The release of such pro-oxidant (Fe^{2+}) is accelerated with the decrease in antioxidants related with DM (Halliwell, 1993).

The most widely accepted hypothesis explaining the association between iron overload and LDL-C oxidation is that high iron level takes part in the formation of highly toxic free radicals such as hydroxide and superoxide anions, which can accelerate the oxidation of lipoproteins (Witztum, 1994; Sulieman *et al*., 2004). Oxidized LDL-C which is generally considered to be generated in vessel walls, exerts several proatherogenic effects including increased synthesis and secretion of adhesion molecules, monocyte chemotaxis and adhesion, cytotoxicity of endothelial cells, enhanced foam cell formation and increased smooth muscle cell proliferation (Steinberg, 1997). These may lead to endothelial dysfunction and macrovascular complications (Saxena *et al*., 2005).

Treatment of diabetic rats with carnosine presented in our investigation, modulate to some extent the alteration in serum lipid profiles and ameliorated the increase in serum iron level induced by DM. These results are in consistent with others who found that carnosine at low concentration effectively reduced triglyceride and cholesterol accumulation in organs from diabetic mice (Lee *et al*., 2005). The hypolipidemic effect of carnosine may be due to glycemic control, improved cortisol level and oxidative stress, modifying carbohydrate and lipid metabolism. Similar results were obtained with other antioxidants such as garlic oil, melatonin, zinc, selenium, vitamins C and E, which have significant lowering effect on total lipids, triglycerides and total cholesterol (Anwar and Meki, 2003; Abdel-Mageed, 2005).

The lowering effect of carnosine on serum iron level of diabetic animals presented in our study is considered one of the mechanisms by which carnosine can protect the biological targets from oxidative stress. It was proved that carnosine has the potential role in the chelation of pro-oxidant transition metals, thereby preventing them from participating in the deleterious damaged reactions (Kang *et al*., 2001). This may suggest that carnosine inhibits the iron released from ferritin through a mechanism of iron ion chelation.

Hepatic fat accumulation is a well-recognized complication of DM. The most common clinical presentation in DM is hepatomegaly and patients have mildly abnormal liver enzymes and normal bilirubin (Levinthal and Tavill, 1999). In agreement with the above report, our data showed a significant statistical elevation

of serum liver enzymes, ALT, AST and SDH in STZ-induced diabetic rats compared to control rats indicating cellular liver damage, however a non significant change in bilirubin level was noted among the experimental groups. The increment in activities of ALT, AST and SDH in serum is mainly due to the leakage of these enzymes from liver cytosol into the blood stream as a result of hepatomegaly (Fatty liver) induced by DM.

Carnosine administration to diabetic animals reflect an improvement of cellular damage as shown by normalization of altered liver enzymes in response to diabetic complications. Our results are consistent with previous studies that administration of some antioxidants (as zinc, selenium, vitamins C and E) to diabetic rats, normalized the elevated activities of such enzymes induced in response to DM (Abdel-Mageed, 2005). Similar protective action of carnosine on liver enzymes against different oxidative stressful conditions producing liver damage (parasitic infections, hypercholesterolemia) was previously proved by Soliman *et al.* (2002, 2004).

The mechanism of hepatoprotective ability of carnosine against membrane damage may be explained by Silaeva *et al.* (1992) who stated that carnosine activation of phagocytic and secretory activities of kupffer cells and increased mitotic activity in hepatocytes, was shown to accelerate liver recovery from carbon tetrachloride (CCL_4), hepatitis in rats. The authors proposed that this pronounced effect of carnosine may be related to its ability to bind to macrophages and stimulates their synthetic and secretory abilities, thus liberating more cellular growth factors, which promote hepatocyte growth and proliferation. The interaction of these factors with lymphocytes strongly activates the natural systems of body immune resistance. Silaeva *et al.* (1992) also attributed the same mechanism of carnosine activating macrophages giving rise to products that in turn, activate lymphocytes to be responsible for restoration of morphology and biochemical function of the liver cells.

Finally, carnosine being one of the endogenous antioxidants, had shown a possible hypoglycemic as well as protective action against metabolic abnormalities induced by DM. This suggests that carnosine may have beneficial effects in type-1 diabetes mellitus that holds the hope of new generation of antidiabetogenic drugs.

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