Journal of Animal and Veterinary Advances 11 (12): 1985-1994, 2012
ISSN: 1680-5593

Protective Effect of Ethanolic Extract of Saffron (Dried Stigmas of *Crocus sativus* L.) on Hepatic Tissue Injury in Streptozotocin-Induced Diabetic Rats

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Abstract: The negative impact of diabetes on the retinal, renal, nervous and cardiovascular systems is well recognized yet little is known about the effect of this disease on the liver. Oxidative stress is currently suggested as a mechanism underlying diabetes mellitus complications. The present study was designed to assess the liver injury as a complication of diabetes mellitus and to evaluate the hepaoprotective properties of ethanolic extract of Saffron (dried stigmas of *Crocus sativus* L.) in streptozotocin-induced diabetes in rats. Aminotransferases, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were measured to determine the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline Phosphatase (ALP) and bilirubin were measured to assess biliary function. Albumin was measured to reflect liver synthetic function. The lipid peroxidation product, Malondialdehyde (MDA) and reduced Glutathione (GSH) content was measured to assess free radical activity in the liver tissues. The enzymatic activities of Glutathione Peroxidase (GSH-Px), Superoxide Dismutase (SOD) and Catalase (CAT) were measured as indicators of antioxidation in liver tissue. Moreover, histopathological observations were assayed at the degree of hepatic injury. For this purpose, male Wistar rats were made diabetic with a single injection of STZ (75 mg kg^{-1} i.p.). Rats were randomly separated into four groups, each containing 10 animals: Group 1, healthy control rats; Group 2 non-diabetic rats were treated with 40 mg kg^{-1} b.w./day intraperitoneal (i.p.) injection of Saffron extract; Group 3, diabetic rats; Group 4, diabetic rats were treated with saffron extract (40 mg kg^{-1} b.w./day, i.p.) for a period of 8 weeks. At the end of the experiment, MDA contents of the liver tissue and serum levels of ALT, AST, AP and bilirubin in Groups 3 were found to be significantly increased as compared to Group 1 ($p<0.05$) and these serum biomarkers of hepatic injury and liver MDA level in Group 4 were significantly decreased as compared to Group 3 ($p<0.05$). The GSH, SOD, CAT and GSH-Px contents of the liver and serum albumin level in Group 3 was significantly decreased as compared to Group 1 ($p<0.05$) and were found to be significantly increased in Group 4 as compared to Groups 3 ($p<0.05$). Histopathological changes were in agreement with biochemical findings. Based on these findings, it is presumed that ethanolic extract of *C. sativus* L. stigma may have the hepatoprotective effect in experimentally induced diabetic rats. Researchers suggest that saffron extract has beneficial effects on antioxidant defence of diabetic liver tissue.

Key words: *Crocus sativus* L., diabetes mellitus, oxidative stress, liver, rat

INTRODUCTION

Diabetes mellitus is a serious metabolic disorder which is a major source of ill health all over the world and its incidence is expected to increase by 5.4% in 2025 (Kim *et al*., 2006). Diabetes mellitus is characterized by hyperglycemia and is associated with disturbances in carbohydrate, protein and fat metabolism which occurs secondary to an absolute (Type I) or relative (Type II) lack of insulin (Alberti and Zimmet, 1998). Type I and II Diabetes Mellitus (T2DM) are influenced by different genetic factors but individuals with either of them are prone to developing complications including nephropathy, retinopathy, peripheral neuropathy and hypertension (Ritz *et al*., 1990; Hendriksen *et al*., 1992; Marjani, 2010; Hameed *et al*., 2002a, b). The negative impact of diabetes on the retinal, renal, nervous and cardiovascular systems is well recognized yet little is known about its effect on the liver (Lipscombe and Hux, 2007; Orasanu and Plutzky, 2009). Diabetes by most estimates is now the most common cause of liver disease in the US and liver disease is an important cause of death

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in Type II diabetes (Marco *et al*., 1999). Thus, patients with diabetes have a high prevalence of liver disease and patients with liver disease have a high prevalence of diabetes. Virtually the entire spectrum of liver disease is seen in patients with Type II diabetes. This includes abnormal liver enzymes, Nonalcoholic Fatty Liver Disease (NAFLD), cirrhosis, hepatocellular carcinoma and acute liver failure (Tolman *et al*., 2007). Chronic mild elevations of transaminases are frequently found in Type II diabetic patients.

Liver is the main effector organ for maintaining plasma glucose levels within narrow limits. The increase of free radical mediated toxicity is well documented in clinical diabetes (Nourooz-Zadeh *et al*., 1997) and streptozotocin-diabetic rats (Wohaieb and Godin, 1987). Hyperglycemia can generate a redox imbalance inside the cells, especially in the liver (Gallou *et al*., 1993). Free radicals result in the consumption of antioxidant defenses which may lead to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation (Vallabhji *et al*., 2001). It was reported that the lipid peroxidation markers are elevated in diabetic rats (Zhang and Tan, 2000).

Oxidative stress occurs when the balance between oxidant and antioxidant systems shifts in favour of the former leading to the generation of free oxygen radicals. Reactive oxygen species are involved in the pathogenesis of many diseases including hypoxia, hypercholesterolemia, atherosclerosis, hypertension, ischemia reperfusion injury and heart failure (Taniyama and Griendling, 2003; Wilcox and Gutterman, 2005; Gupta *et al*., 2008; Siddique and Afzal, 2008; Hosseini *et al*., 2009; Nourmohammadi *et al*., 2010; Momtaz and Abdollahi, 2012; Karim *et al*., 2011; Sohail *et al*., 2011). It has been shown that patients with diabetes mellitus have increased oxidative stress and impaired antioxidant defense systems which appear to contribute to the initiation and progression of diabetes-associated complications (Maritim *et al*., 2003). There is convincing experimental and clinical evidence that the generation of reactive oxygen species is increased in both types of diabetes. Normally, the level of oxidative stress is modulated by antioxidant defense systems (Saxena *et al*., 1993). Diabetes-linked alterations in antioxidant defense system enzymes such as catalase, glutathione peroxidase, superoxide dismutase have been demonstrated (Maritim *et al*., 2003). However, lipid peroxidation and antioxidant status of hepatic tissue were studied by in experimental diabetes (Feillet-Coudray *et al*., 1999).

However, when the liver fails, there is no equivalent form of management such as hemodialysis or retinal photo-coagulation. Thus, diabetic hepatopathy is potentially less common, it may be appropriate for addition to the list of target organ conditions related to diabetes such as glomerulopathy, retinopathy and neuropathy. However, annual screening for liver disease might be accomplished by means of a simple biochemical analyte such as alanine aminotransferase (Athyros *et al*., 2006).

Antidiabetic agents have generally been shown to decrease the levels of serum biomarker of hepatic injury (Harris, 2005) but these agents can produce serious side effects (Akhtar and Iqbal, 1991). Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for hyperglycemia and liver toxicity. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely even when their biological active compounds are unknown (Gupta *et al*., 2005). Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety.

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities (Frei and Higdon, 2003). Herbal formulations with a simultaneous antioxidant effect would thus be more useful in the management of diabetes mellitus. Their use alone or in combination with oral hypoglycemic agents or insulin may help in the better control of blood glucose level in diabetic subjects. Natural antioxidants strengthen the endogenous antioxidant defenses from Reactive Oxygen Species (ROS) and restore and optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, Saffron can rightly be mentioned as a plant of considerable interest.

Saffron (dried stigmas of *Crocus sativus* L.) is the world's most expensive spice and genuine Saffron is worth its weight in gold. This plant belongs to the Iridaceae family and widely cultivated in Iran and other countries such as India and Greece. As a therapeutic plant, Saffron is considered an excellent aid for stomach ailments and an antispasmodic, helps digestion and increases appetite. It has been reported that *C. sativus* has hypolipaemic, anti-inflammatory, antioxidant and anticancer effects. Moreover, according to Commission E, *C. sativus* is applicable for treatment of nervous disorders, spasms and asthma (Abdullaev, 2002; Abe and Saito, 2000; Rios *et al*., 1996). Aqueous saffron extract and its active constituent, crocin are useful agents for the prevention of renal Ischemia-Reperfusion (IR)-induced oxidative injury in rats (Hosseinzadeh *et al*., 2005).

Furthermore, Saffron extract protects against oxidative damage in rat primary hepatocytes. It also, suppresses aflatoxin B1-induced hepatotoxic lesions and has a modulatory effect on aflatoxin B1, cytotoxicity. It also has a protective effect on the bladder toxicity induced by cyclophosphamide (Giaccio, 2004). Recently, it was reported that the saffron extract, crocin and safranal exhibited significant radical scavenging activity and thus antioxidant activity (Assimopoulou *et al*., 2005). Antihyperglycemic and antidiabetic effects of ethanolic extract of Saffron is previously reported (Tabrizi *et al*., 2008; Mohajeri *et al*., 2009).

Considering the anti-hyperglycemic properties and antioxidant activities of Saffron this study was designed to evaluate the hepatoprotective effects of Saffron ethanolic extract in streptozotocin-induced diabetes of rats. To the knowledge this is the first investigation on the effect of saffron extract on the antioxidant status of liver tissue in experimental diabetic rats. Researchers report on the effect of saffron ethanolic extract on liver tissue oxidative parameters in rats with streptozotocin-induced diabetes. In this context, Saffron can rightly be mentioned as a plant of considerable interest.

MATERIALS AND METHODS

Chemicals: Streptozotocin was from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade. All chemicals used in this study were of analytical grade and obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

Plant: The Saffron used in this study was dedicated by Novin Zaferan Co. (Mashhad, Iran) and was identified by the Department of Cultivation and Development of Institute of Medicinal Plants, Tehran, Iran.

Preparation of the extract: In the maceration method, 10 g of stigmas were macerated in 500 mL ethanol (80 v/v) for 3 days. The mixture was subsequently filtered and concentrated under reduced pressure at 40°C. The extract yield was 51% w/w.

Induction of diabetes mellitus: Diabetes was induced by intravenous injection of streptozotocin (Sigma, St. Louis, Mo, USA) into the tail vein at a dose of 65 mg kg^{-1} body weight. STZ was extemporaneously dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. After 18 h, animals with fasting blood glucose levels >16.5 mmol L^{-1} were considered diabetic and then included in this study (Eddouks *et al*., 2005).

Animal treatment: Forty healthy male Wistar rats (about 180-200 g body weight) were used for this study. All animals were conditioned at room temperature at a natural photoperiod for 1 week before experiment execution. A commercial balanced diet and tap water *ad libitum* were provided. The duration of experiment was 8 weeks. The rats were randomly divided into 4 groups (10 rats each) as the following: Group 1, healthy control rats received Isotonic Saline Solution (ISS, 10 mL kg^{-1}) intraperitoneally; Group 2 non-diabetic rats were treated with 40 mg kg^{-1} b.w./day intraperitoneal (i.p.) injection of saffron extract; in Group 3, diabetic rats administered by ISS (10 mL kg^{-1}) was given through Intraperitoneal (IP) route; Group 4, diabetic rats were treated with Saffron extract (40 mg kg^{-1} b.w./day, i.p.) for a period of 8 weeks. The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment.

Biochemical factors evaluation: At the end of experiment, blood samples were collected from the retro-orbital plexus and the sera prepared through centrifuging at 2500× g for 15 min at 30°C. Serum biomarkers of liver function including ALT, AST (Reitman and Frankel, 1957), ALP (Kind and King, 1954), albumin (Lowry *et al*., 1951) and total bilirubin (Malloy and Evelyn, 1937) were measured using commercially available kits.

Microscopic studies: The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment. A small piece of hepatic tissue from the anterior portion of the left lateral lobe was removed for histological analysis. The sample was fixed by immersing it in 10% neutral-buffered formalin. The sample was then embedded in paraffin, sliced into 5 μm sections and stained with hematoxylin-eosin for blinded histological assessment. The degree of liver tissue injury was evaluated semiquantitatively according to the method reported by Jamshidzadeh *et al*. (2008). The stained 5 μm sections were graded as follows: 0, absent; 1, minimal; 2, mild; 3, modest and 4, severe. The histological changes were evaluated in nonconsecutive, randomly chosen x200 histological fields using light microscope, NIKON ECLIPSE E200.

Measurement of antioxidant activity: The rat's liver was removed immediately and washed in normal saline and homogenate 10% prepared in 1.15% w/v of potassium chloride. The homogenate was centrifuged in 7000×g for 10 min at 4°C and supernatant were used for measurement of oxidative stress by estimation of reduced Glutathione (GSH) and determination of Malondialdehyde (MDA) as

well as Antioxidant Enzymes (AOE) such as Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GSH-Px). GSH, MDA, SOD, CAT and GSH-Px were measured by using commercially available kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Reduced Glutathione (GSH) content was determined according to Sedlack and Lindsay (1968). GSH reacts with 5, 5'-dithiobis-2-nitrobenzoic acid and the absorbance spectra of the product have a maximum absorbance at 410 nm. The results were expressed as μmol/g wt. Liver homogenate MDA levels were expressed as nmol MDA per mg protein and antioxidant activity was expressed as arbitrary units per mg protein. Degree of lipid peroxidation in liver tissue homogenates was determined in terms of Thiobarbituric Acid Reactive Substances (TBARSs) formation by following the protocol of Esterbauer and Cheeseman (1990). SOD activity was measured by Nishikimi Method (Nishikimi *et al*., 1972) and was modified by Kakkar Method (Kakkar *et al*., 1984). Each unit of SOD activity was determined as required enzyme concentration for prohibition of creation color at 1 min under study conditions. CAT activity was measured by Claiborne Method (Claiborne, 1985) and was based on hydrogen peroxide breakdown. GSH-Px activity was measured by Rotruck Method (Rotruck *et al*., 1973) and was expressed as micromole of GSSG/min/mg of protein, based on reaction:

$$2H_2O + GSSG \rightarrow H_2O_2 + 2GSH$$

Statistical analysis: The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 13.0 was used for statistical analysis. All data are presented as mean±SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. The Kruskal-Wallis test followed by Mann-Whitney U post-test was used for the analysis of degree of histopathological liver injury $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 shows the effects of ethanolic Saffron extract on the serum levels of markers of liver injury (ALT, AST, ALP and bilirubin) in diabetic rats. ALT, AST, ALP and bilirubin serum contents in Groups 3 was found to be significantly increased as compared to Group 1 ($p<0.05$) and these parameters in Group 4 were significantly decreased as compared to Group 3 ($p<0.05$). The albumin serum level in Group 3 was significantly decreased as compared to Groups 1 ($p<0.05$) and this parameter was significantly increased in Group 4 as compared to Group 3 ($p<0.05$).

Table 2 shows the effects of ethanolic Saffron extract on antioxidative activity in liver tissue of diabetic rats. MDA contents of the liver tissue in Groups 3 was found to be significantly increased as compared to Group 1 ($p<0.05$) and liver MDA level in Group 4 were significantly decreased as compared to Group 3 ($p<0.05$). The GSH, SOD, CAT and GSH-Px contents of the liver in Group 3 were significantly decreased as compared to Groups 1 ($p<0.05$) and GSH, SOD, CAT and GSH-Px activity were increased in Group 4 as compared to Group 3 ($p<0.05$).

Table 1: Comparison of the effect of ethanolic Saffron extract on serum markers of liver tissue injury among the experimental groups (mean±SEM)

		Biochemical parameters				
Groups	Treatments	Alanine aminotransferase (U L^{-1})	Aspartate aminotransferase (U L^{-1})	Alkaline phosphatase (U L^{-1})	Total serum bilirubin (Mg dL^{-1})	Albumin (g dL^{-1})
1	Healthy control rats	54.82±2.36	68.90±2.31	194.87±9.00	0.81±0.03	4.38±0.32
2	Non-diabetic rats+Saffron extract	55.90±2.75	67.21±2.84	203.55±8.80	0.87±0.06	4.30±0.56
3	Diabetic rats	76.45±3.95[a]	101.19±3.96[a]	270.20±10.5[a]	1.21±0.08[a]	3.17±0.25[a]
4	Diabetic rats+Saffron extract	51.30±2.64[b]	70.19±3.11[b]	214.73±9.90[b]	0.89±0.04[b]	4.29±0.41[b]
	ANOVA	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000

Table 2: Comparison of the effect of ethanolic saffron extract on liver MDA and GSH contents and antioxidant enzymes activities among the experimental groups (mean±SEM)

		Biochemical parameters				
Groups	Treatments	GSH (μg mg^{-1} protein)	MDA (nmoL g^{-1} protein)	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GPX (U mg^{-1} protein)
1	Healthy control rats	8.92±0.61	3.58±0.14	13.57±0.51	62.11±3.40	21.81±1.13
2	Non-diabetic rats+Saffron extract	8.14±0.57	3.65±0.19	12.76±0.83	61.92±2.35	20.68±1.32
3	Diabetic rats	5.89±0.69[a]	5.42±0.21[a]	8.65±0.60[a]	49.64±1.18[a]	17.45±0.66[a]
4	Diabetic rats+Saffron extract	7.74±0.51[b]	4.15±0.41[b]	11.15±0.47[b]	57.93±2.55[b]	19.75±1.10[b]
	ANOVA	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000

$p<0.05$; [a]Compared to Group 1 and [b]Compared to Group 3

Pathologically, liver histological structure was normal in healthy control group (Fig. 1a). In Group 2 also there were no pathological changes so that hepatic lobular structure seemed quite normal (Fig. 1b). In Group 3, iabetic rats showed fatty changes in centrilobular portions of the livers (Fig. 1c). Finally in Group 4, ethanolic Saffron extract prevented the pathologic changes and no considerable fatty change was observed (Fig. 1d). Quantitative microscopic results of experimental rats are shown in Table 3.

In the current study, results of biochemical and histopathological assessments, reflects liver injuries in rats with streptozotocin-induced diabetes. Significant elevations in markers of liver injury (ALT, AST and ALP) as well as bilirubin and significant decrease in serum albumin in streptozotocin-induced diabetic rats were observed in comparison with normal healthy rats. This result is consistent with the findings reported by Ramesh *et al*. (2007). Individuals with Type II diabetes have a higher incidence of liver function test abnormalities than individuals who do not have diabetes (Harris, 2005). This finding is also, in line with the results.

Table 3: Effect of ethanolic saffron extract on hepatic injuries of diabetic rats (mean±SEM)

Groups	Degree of liver tissue injury	The Kruskal-Wallis test
1 (healthy control rats)	0.0±0.0	p<0.001
2 (non-diabetic rats+Saffron extract)	0.0±0.0	-
3 (diabetic rats)	2.38±0.25[a]	-
4 (diabetic rats+Saffron extract)	0.45±0.12[b]	-

0 = Without injury, 1 = Minimum injury, 2 = Mild injury, 3 = Moderate injury, 4 = Sever injury (n = 10), [a]Compared to Group 1 and [b]Compared to Group 3

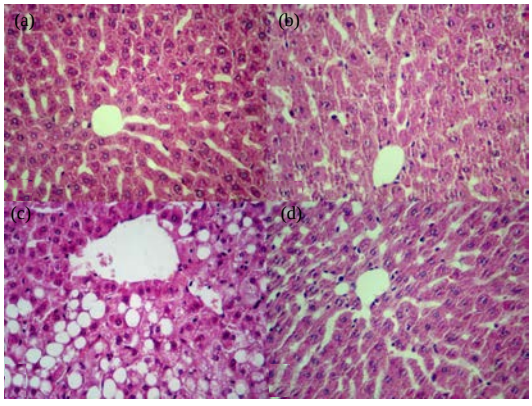

Fig. 1: Microscopic appearance from liver tissues of the experimental rats (H and E, 100x); a) healthy control rat liver showing normal hepatocytes; b) non-diabetic+Saffron extract treated rat liver shows normal appearance; c) Diabetic rat liver showing macrovesicular fatty change in centrilobular portions d) Diabetic+Saffron extract treated rat liver showing no fatty change

The data of this study also, revealed that daily treatment of Saffron extract markedly improves biochemical and histopathological status of rats with streptozotocin-induced diabetes.

Liver Function Tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin and albumin. Hepatocellular damage causes release of these enzymes into circulation. Increase in serum levels of AST shows hepatic injuries similar to viral hepatitis infarction and muscular damages. ALT which mediates conversion of alanine to pyruvate and glutamate is specific for liver and is a suitable indicator of hepatic injuries. Increased levels of these enzymes are an indicator of cellular infiltration and functional disturbance of liver cell membranes (Drotman and Lawhan, 1978). In addition, ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites (Mehana *et al*., 2012). On the other hand, bilirubin and albumin values are associated with the function of hepatic cells (Muriel *et al*., 1992).

Return of the above enzymes to normal serum values following Saffron extract treatment may be duo to prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration (Thabrew *et al*., 1987). Effective control of bilirubin and albumin shows early improvement of functional and secretory mechanism of hepatic cells.

In this study, histopathological evaluation of liver tissues showed fatty changes in centrilobular portions of the livers in diabetic rats. These results are in line with the findings reported by Ramesh who observed the hepatoprotective action of Umbelliferone in streptozotocin-induced diabetic rats (Ramesh *et al*., 2007). With Saffron treatment in diabetic rats no considerable fatty change were observed indicating the protective effect of ethanolic Saffron extract against hepatic complications of diabetes. However, pathologic findings are matched with biochemical results.

In the present study, significant decline in GSH level and antioxidant enzymes activity as well as increased lipid peroxidation in the liver tissue of rats reflects oxidative stress of the liver in experimental diabetes. These results are in line with the findings reported by Feillet-Coudray *et al*. (1999) who observed that STZ-induced diabetes in rat accompanied with an increase in the susceptibility to lipid peroxidation. The data of this study also revealed that daily treatment of Saffron extract markedly improves antioxidant status of liver tissue of rats with streptozotocin-induced diabetes.

GSH (an important part of the non-enzymatic antioxidant system) is a major non-protein thiol in living organisms which plays a central role in co-ordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system can lead to serious consequences. Elevation in MDA level and reduction in GSH stores of liver tissue of diabetic rats suggest that oxidative stress due to free-radical damage is one of the possible mechanisms in the pathophysiology of diabetic hepatopathy. On administration of Saffron extract, the MDA levels have decreased and the GSH levels have increased. This indicates that in the presence of Saffron extract there is an improvement in the oxidative stress. Increased oxidative stress in the tissues of streptozotocin diabetic rats was similarly reported. This was said to be a contributory factor in the development of the complications of diabetes (Kakkaar *et al*., 1995; Curcio *et al*., 1995). It was observed in that study that GSH administration reverses these effects.

Free radicals are the chemically most reactive substances in the human or animal organism (Halliwell and Gutteridge, 2002; Fridovich, 1995; Nicotera and Orrenius, 1997). The unbalance between formation and detoxification of free radical species results in the progression of oxidative stress (Sies, 1991). Oxidative stress causes serious damage in biomolecules such as proteins (Goldstein *et al*., 1994; Berlett and Stadtman, 1997; Hazen *et al*., 1997; Leeuwenburgh *et al*., 1999; Stadtman, 1990), lipids (Nacitarhan *et al*., 1995; Ziouzenkova *et al*., 1998) and nucleic acids (Murata *et al*., 1998; Marnett, 2000) and leads to the development of a wide spectrum of serious diseases (Halliwell *et al*., 1992) some types of neoplasia (Toyokuni, 1996; Mitchell *et al*., 2003) inflammation processes (Pavlick *et al*., 2002), ischaemic and reperfusion states (Ghoneim *et al*., 2002), acute pancreatitis (Urunuela *et al*., 2002), atherosclerosis (Noguchi, 2002) or diabetes mellitus (Vozar, 1998) and diabetic complications (Halliwell and Gutteridge, 1989a).

SOD, CAT and GPx constitute a mutually supportive team of defense against ROS. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady-state level of O_2^-. In hyperglycaemia, glucose undergoes autooxidation and produces superoxide and it produces free radicals that in turn lead to lipid peroxidation in lipoproteins. CAT is localized in the peroxisomes or the microperoxisomes which catalyses the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage produced by H_2O_2. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form Glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In this study, decline in the activities of these enzymes in liver tissue of streptozotocin-induced animals and attainment of near normalcy in Saffron extract treated rats indicate oxidative stress elicited in hepatic tissue of diabetic rats had been nullified due to the effect of the extract. This observation perfectly agrees with those of Krishnakumar *et al*. (1999) who demonstrated hypoglycaemic and antioxidant activity of *Salacia oblonga* extract in streptozotocin induced diabetic rats.

Evidence suggests that oxidative stress and free radicals play an important role in the pathogenesis of diabetes mellitus and diabetic complications (Halliwell and Gutteridge, 1989b). Liver is one of the most important organs that maintains blood glucose levels within normal limits thus enhancement of blood glucose yield to imbalance of oxidation-reduction reactions in hepatocytes so that, hyperglycemia through increasing in AGEs (Advanced Glycation End products) facilities free radicals production through disturbance in ROS production (Reactive Oxygen Species) such as SOD and CAT (Cameron *et al*., 2005; Kalia *et al*., 2004; Jandeleit-Dahm *et al*., 2005). Hence, it reveals that diabetic hepatic injuriy results from several agents and is not controllable only via inhibition of hyperglycemia (Liu *et al*., 2008). Namely although, in early stages of diabetes, tissues injuries are in association with hyperglycemia but its progress in latter stages is not related to hyperglycemia (Vestra and Fioretto, 2003). Therefore, monitoring of blood glucose levels solely is not sufficient in retarding diabetes complications. Thus, a suitable drug must have both antioxidant and blood glucose decreasing properties (Ramesh and Pugalendi, 2006).

Saffron extract consists of many compounds such as α-crocin, a water soluble carotenoid, crocetins, picrocrocin and safranal (Morimoto *et al*., 1994). Saffron stigmas and its active constituents, crocetin and crocin have established antioxidant effects (Rios *et al*., 1996; Abdullaev, 1993). These carotenoids scavenge free radicals, especially superoxide anions and thereby may protect cells from oxidative stress (Bors *et al*., 1982). There was also, a constant decrease in lipoprotein oxidation susceptibility in healthy individuals after administration of 50 mg of saffron twice a day (Verma and Bordia, 2008). In addition saffron contains proteins, sugars, vitamins (especially riboflavin), amino acids, minerals and gums (Rios *et al*., 1996). Thus, Saffron extract is a complex mixture of different compounds that often act in a synergistic fashion and exert their full beneficial effect as total extract.

CONCLUSION

Researchers observed that ethanolic Saffron extract improved serum biomarkers of liver tissue injury and histopathologic properties of this organ. It is therefore likely that ethanolic Saffron extract is prophylactic against diabetic complications and ameliorates diabetic hepatopathy through its antioxidant potential. Therefore, ethanolic Saffron extract consumption may be associated with a reduced risk of diabetic hepatopathy in apparently healthy individuals. Moreover, researchers speculate that the observed effects of ethanolic Saffron extract are primarily due to the promotion of antioxidant status in peripheral tissues such as liver.

Hyperglycemia is the primary symptom of diabetes and is blamed for the complications of diabetes because elevated glucose concentration directly injures cells and induces lipid peroxidation (Davi *et al*., 2005). Whether this reflects oxidative stress induced liver injury or direct glycemic injury of liver remains to be determined.

ACKNOWLEDGEMENT

This research was supported by a Research Fund of the Ahar Branch, Islamic Azad University.

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