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Protective Role of Wheat Germ Oil against Hyperglycemia and Hyperlipidemia in Streptozotocin Induced Diabetic Rats

¹Basma H. Merghani, ²Walaa F. Awadin, ¹Yousef Y. Elseady and ¹S.A. Nabil Abu-Heakal

¹Department of Animal Physiology, Faculty of Veterinary Medicine, Mansoura University, Egypt

²Department of Pathology, Faculty of Veterinary Medicine, Mansoura University, Egypt

Corresponding Author: Walaa F. Awadin, Department of Pathology, Faculty of Veterinary Medicine, Mansoura University, P.O. Box 35516, Egypt Tel: +201011610725 Fax: +20502379952

ABSTRACT

In this study, the possible protective effects of oral administration of wheat germ oil (WGO) at 2 doses (300 and 500 mg kg⁻¹ b.wt.) in normal, non-diabetic and streptozotocin (STZ) induced diabetic rats. Six groups of male *Sprague Dawley* rats were used; group 1: control negative, group 2: control negative treated with 300 mg kg⁻¹ b.wt., WGO, group 3: control negative treated with 500 mg kg⁻¹ b.wt., WGO, group 4: STZ diabetic rats, group 5: STZ diabetic rats treated with 300 mg kg⁻¹ b.wt., WGO and group 6: STZ diabetic rats treated with 500 mg kg⁻¹ b.wt., WGO. Blood and Serum analyses were carried out after 6 weeks (end of the experimental period) to determine the serum insulin hormone, fasting blood glucose levels (FBG), glycated hemoglobin percent (HbA1c), serum levels of Total Cholesterol (TC), triglycerides (TGs), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C), TGs/HDL-C ratio and TC /HDL-C ratio. Liver tissue homogenate was used for evaluation of malondialdehyde (MDA) level. Microscopic examination of liver and pancreas, besides, estimation of caspase enzyme immunolabeling in both organs were made in all groups. Oral administration of 500 mg kg⁻¹ b.wt., WGO to non-diabetic rats significantly decreased HbA1c%, serum lipid profile, Atherogenic Index (AI), liver MDA and significantly (p>0.05) increased HDL-C level when compared to control negative group. In STZ induced diabetic rats, high dose of WGO was more effective than lower dose in correcting the significantly (p>0.05) elevated FBG, HbA1c, serum lipid profile, MDA levels and the significantly reduced levels of insulin hormone and HDL-C. The histopathological and immunohistochemical examination confirmed the biochemical results. About 500 mg kg⁻¹ b.wt., WGO has anti-hyperglycemic, anti-hyperlipidemic and has an antioxidant activity.

Key words: Wheat germ oil, diabetes, insulin, histopathology, antioxidants

INTRODUCTION

Diabetes Mellitus (DM) considered one of the most common chronic diseases worldwide and recognized as one of the leading causes of morbidity and mortality (American Diabetes Association, 2010). More than 347 million people have diabetes (Danaei *et al.*, 2011). Diabetes caused by either reducing insulin secretion due to damage in beta cells of pancreas (type I) or decreased responsiveness of the peripheral insulin receptors in peripheral tissues (type II diabetes) (Poitout, 2008). Diabetic people have hyperglycemia, glucosuria, polyphagia, polydipsia and

polyuria (Moussa, 2008). Due to the prolonged period of hyperglycemia, diabetes leads to irreversible tissue damage such as retinopathy, nephropathy, arteriosclerosis and vascular damage (Luitse *et al.*, 2012).

Streptozotocin (STZ) is a cytotoxic substance obtained from the soil microbes, *Streptomyces achromogenes* that induces DM in experimental animals (Shrilatha and Muralidhara, 2007). The STZ penetrates the beta cells (β -cells) of pancreas via the low affinity glucose protein 2-transporter and breaks the DNA strand in β -cells causing a drastic reduction in insulin production, amendment of glucose level in the blood (Vikas *et al.*, 2011). Several studies have shown that cytotoxic effects of STZ were produced by the releasing of Reactive Oxygen Species (ROS) or the intracellular liberation of Nitric Oxide (NO) (Szkudelski, 2001) and the imbalance between plasma oxidant and antioxidant content, DNA alkylation (Elsner *et al.*, 2000).

Wheat has been called the staff of life and the oil from the germ of the wheat has long been used by people with a variety of health concerns. It is a valuable anti-diabetic because of the high level of polyunsaturated acids and vitamin E (Gruenwald *et al.*, 2004). Wheat Germ Oil (WGO) can help to reduce oxidative stress (Alessandri *et al.*, 2006). Also contains policosanol, a substance that can be helpful in lowering raised blood sugar and/or total cholesterol levels (Irmak and Dunford, 2005). The WGO was known to be the richest natural source of alpha, beta and gamma-tocopherols and tocotrienols (Hassanein and Abedel-Razek, 2009). The WGO induces the tocopherol-mediated redox system and inhibits the synthesis of eicosanoid (Prostaglandins), which activates the lipid peroxidation (LPO) process (Chang *et al.*, 2010). The WGO also contains fat-soluble carotenoids such as lutein, zeaxanthin and beta-carotene, which have antioxidant effect (Leenhardt *et al.*, 2008).

The objective of the present investigation was to evaluate the effect of oral administration of WGO as a natural antioxidant at two doses (300 and 500 mg kg⁻¹ b.wt.) on serum insulin hormone, Fasting Blood Glucose (FBG), glycated hemoglobin percent (HbA1c), serum total lipid profile, lipid peroxidation, microscopic examination of liver and pancreas, besides, levels of caspase enzyme immunolabeling in both organs in control negative and STZ induced diabetic rats.

MATERIALS AND METHODS

Materials

Experimental animals: Thirty mature *Sprague dawley* male rats weighting 130-160 g b.wt., were used. Animals were purchased from the animal house in Helwan-Egypt and were housed in separate cages in department of physiology, Mansoura University, Faculty of Veterinary Medicine. Animals were left for one week to acclimatize the place. Rats were kept in cages, five rats per cage, in a controlled environment and maintained under a 12 h light: dark cycle, 24°C ($\pm 3^\circ\text{C}$) and 50-70% humidity. Rats were provided with standard diet and water *ad-libitum*. Animals received human care in compliance with the guidelines of animal care of the National Institutes of Health (NIH) and all animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Egypt. Registration number (09/189).

Ration

Normal control ration: Normal control ration constituents were obtained from Faculty of Agriculture, Mansoura University. The ration was pelleted composed of (Ground yellow corn 71.2%, Wheat bran 7%, Soya bean meal 5%, Corn gluten 9%, Limestone 2%, Sodium chloride 0.5%, Di-calcium phosphate 2%, Premix 0.3% and Molasses 3%). The ingredients of the basal control ration to meet the recommended nutrients requirements for growth of laboratory rats according to NRC (1995).

Wheat germ oil: The WGO was purchased from Sedico Pharmaceutical Company (Extra-1000-Sedico) in the form of soft gelatin capsules. Each capsule contains WGO 1000 mg, given to the animals every day by oral gavages using stomach tube for six weeks at two dose levels (300 and 500 mg kg⁻¹ b.wt.).

Equipment

Glucometer: Measurement of blood glucose levels in blood samples taken from tip of tail under anesthesia and stomach tube for oral gavages of WGO.

Chemicals: Diethyl ether for anesthesia, STZ was used for experimental induction of diabetes in rats in the form of vial containing 1 g STZ powder, cold phosphate buffer saline (50 Mm potassium phosphate, pH 7.5) for liver tissue homogenate, cold 0.1 M citrate buffer for STZ dissolving, EDTA as anticoagulant, commercial kits for analysis of HbA1c, serum fasting insulin, triglycerides (TGs), Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C) and liver malondialdehyde (MDA).

Methods

Induction of experimental diabetes in rats by STZ: For induction of type 1 DM. Rats were fasted overnight then injected intraperitoneally (IP) with 50 mg STZ previously dissolved in freshly prepared cold 0.1 M citrate buffer (0.1 M citric acid, 0.1 M trisodium citrate, PH is 4.5) according to Mohammadi *et al.* (2013). The animals were given 5% glucose water for 24 h following STZ injection to prevent initial drug induced hypoglycemic mortality. After IP injection of STZ, the rats were fasted for 14 h, lightly anaesthetized with diethyl ether and blood samples were collected through cutting the tip of tail. The hyperglycemia was confirmed by measuring fasting blood glucose levels using glucometer (One touch technology). Animals showed symptoms of polydipsia, polyuria and fasting blood glucose higher than 250 mg dL⁻¹ were considered diabetic and were included in this investigation. Treatment with WGO was started 3 days after the induction of diabetes and continued for 6 weeks the duration of experiment.

Experimental design: Thirty rats were randomly divided into six groups (5×6). The experimental design was as the following, group (1) control negative, groups (2 and 3) control negative rats orally gavages with WGO at two doses (300 and 500 mg kg⁻¹ b.wt), respectively, group (4) STZ induced diabetic rats and groups (5 and 6) STZ treated diabetic rats with two doses of WGO (300 and 500 mg kg⁻¹ b.wt.), respectively.

Sampling

Blood samples: At the end of the experimental period, all rats were fasted overnight, anaesthetized using diethyl ether and blood samples were withdrawn from retro orbital plexus into heparinized capillary tube. Each blood sample was immediately divided into two sterile, dry, capped tubes: (1) One tube contained Ethylene Diamine Tetra Acetic Acid (EDTA) tube (as anticoagulant) for estimation of HbA1c percentage. (2) Second tube was empty for serum separation and the blood kept inside in a vertical position at room temperature then centrifuged at 3000 g for 15 min. A clear, straw colored serum sample aspirated by automatic pipette and transferred into clean, dry, labeled tubes and kept at -20°C for subsequent biochemical analysis.

Tissue sampling: Rats were dissected at the end of the experimental period. Pancreas and liver were collected from each rat, washed with normal physiological saline to clean the remaining blood.

One gram of the liver tissue was used for preparation of liver homogenate for analysis of liver MDA. The remaining parts of the liver and the pancreas were fixed in 10% formalin for histopathological examination.

Preparation of liver tissue homogenate: One gram of liver tissue was homogenized in 9 mL cold phosphate buffer saline (50 Mm potassium phosphate, pH 7.4). After centrifugation at 4000 g for 15 min at 4°C, about 3 mL supernatant was aspirated, collected into separate Eppendorf tubes and stored at -20°C for further biochemical analysis of liver MDA.

Biochemical analysis: Serum insulin was kindly measured by enzyme linked immunosorbent assay (ELIZA) in the Faculty of Medicine, Mansoura University using immuolite 2000 device, according to Sapin (2007), glycated hemoglobin (HbA1C) was determined using a commercial kit according to Hanas and John (2010), serum lipid profile was estimated using commercial kits according to Lalouschek *et al.* (2003). Atherogenic Index (AI) was calculated using the logarithm of the ratio of triglycerides to high density lipoprotein ($\log[\text{TG}/\text{HDL-C}]$) according to what previously described by Dobiasova and Frohlich (2001). Liver tissue MDA (nmol g^{-1} tissue) was assessed by using commercial kit according to Mateos *et al.* (2005).

Histopathological and immunohistochemical examination: Fixed specimens were processed routinely until embedding in paraffin wax. Paraffin sections of 5 μm thickness were cut and picked up on uncoated slides, dried, deparafinized, rehydrated with graded alcohol, washed and stained with H and E according to Bancroft *et al.* (1996). Sections of pancreas and liver from all groups were picked up on coated slides, dried, dewaxed, washed in phosphate buffer saline, drained and incubated with citrate buffer (pH-6) for 30 min. at 94°C. After endogenous peroxidase was inactivated by incubation with 0.3% hydrogen peroxide, the sections were incubated with a 1:120 dilution of primary polyclonal rabbit antibody against caspase-3 (Thermo Fisher Scientific) for 24 h at room temperature in a dark chamber. After washing the slides in PBS, the slides were incubated with LSAB 2 kit/HRP (Thermo Fisher Scientific) and developed using diaminobenzidine (Dako Company) as the substrate to produce a brown stain then counter stained with Mayer's hematoxylin. The positive signal of caspase-3 protein was brown granular mass that could be used to trace this protein. Histological and immunohistochemical changes were examined by light microscopy (binocular, Olympus). Images were taken using Digital camera (Canon 5 mega pixels, 3.2x optical zoom). The positive signal of caspase-3 protein was brown granular mass that could be used to trace this protein. A total of 25 fields per group (five fields per section and five sections per group) were randomly selected and examined. A semi-quantitative score from 0-3 was given to assess the intensity of this positive signal 0; when negative, (1) If the antigen was located infrequently, (2) If the antigen was moderately distributed in the field and (3) If the antigen was widely distributed in the field.

Statistical analysis: All the data of the animal experiments were expressed as Means $\pm$ SEM. Statistical analysis of data was carried out by software SPSS program package version 17 (SPSS., 2004) using the one-way analysis of variance ANOVA followed by Duncan's Multiple Range Test (DMRT) for testing the significance differences between variables. Moreover, the correlation[®] was determined between parameters. Results were considered significant only at the

level of p (0.05) or less. The positive signal of caspase-3 protein was tested by student T test to compare between control and experimental animals at each appropriated time. The means were considered significantly different when $p < 0.05$.

RESULTS

Fasting blood glucose, glycated hemoglobin%, serum insulin, Lipid profile and MDA levels estimation: The oral administration of rats in group 3 with the high dose of WGO ($500 \text{ mg kg}^{-1} \text{ b.wt.}$) significantly ($p < 0.05$) decreased HbA1c%, serum lipid profile (TC, TGs, LDL-C, VLDL-C, the ratios of TGs/HDL-C, TC/HDL-C), AI and liver MDA and significantly ($p < 0.05$) increased HDL-C level when compared to group 1. Non-significant difference was detected in serum insulin levels between groups 1, 2 and 3 (Table 1). On the other hand, STZ induced diabetic rats in group 4 showed significant ($p < 0.05$) increases in FBG, HbA1c%, serum levels of TC, TGs, LDL-C, VLDL-C, ratios of TC/HDL-C and TGs/HDL-C and AI and significant decreases in serum insulin level and HDL-C when compared to groups 1, 2 and 3. Liver MDA level was the highest in STZ induced diabetic rats in group 4 (Table 1). The treatment of STZ induced diabetic rats with $500 \text{ mg kg}^{-1} \text{ b.wt.}$, WGO was better than $300 \text{ mg kg}^{-1} \text{ b.wt.}$, WGO in decreasing levels of FBG, HbA1c, serum lipid profile and MDA and reducing serum insulin and HDL-C levels (Table 1).

Histopathology

Liver: Histopathological examination of livers from groups 1-3 revealed normal histological structure of hepatic lobule consisting of sinusoidal cords of hepatocytes with central vein and portal tracts. The portal tracts showed portal triad with portal vein, hepatic artery and bile duct. Liver sections from untreated diabetic rats in group 4 showed congestion, marked perivascular fibrosis, mononuclear cells (MNCs) infiltration and hepatocytes degeneration. Liver sections from diabetic rats supplemented with WGO ($300 \text{ mg kg}^{-1} \text{ b.wt.}$) showed mild hepatocytes degeneration, individual cell death and MNCs infiltration in portal area. Meanwhile, liver sections from diabetic rats supplemented with WGO ($500 \text{ mg kg}^{-1} \text{ b.wt.}$) appeared as control (Fig. 1a-d). Immunohistochemistry revealed the increased positive signal for caspase-3 in liver sections from rats in group 4 than in other groups (Fig. 2a-d and Fig. 3).

Pancreas: Histopathological examination of pancreas from groups 1-3 revealed normal arrangement of the islets of Langerhans of various sizes scattered throughout the exocrine tissue

Table 1: Effect of WGO (300 and $500 \text{ mg kg}^{-1} \text{ b.wt.}$) on serum lipid profile, blood glucose, glycated hemoglobin percentage and liver MDA in different experimental groups

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Insulin (mlu mL^{-1})	12.50 ± 0.87^a	11.11 ± 0.23^a	12.43 ± 0.64^a	2.40 ± 0.23^d	6.37 ± 0.20^c	9.14 ± 0.45^b
FBG before treatment (mg dL^{-1})	85.00 ± 2.52^b	87.66 ± 0.33^b	88.67 ± 1.76^b	511.00 ± 19.55^a	489.00 ± 11.00^a	474.00 ± 38.08^a
FBG after treatment (mg dL^{-1})	86.66 ± 3.52^d	79.00 ± 8.32^d	72.00 ± 7.02^d	495.66 ± 4.33^a	372.00 ± 8.08^b	224.66 ± 8.61^c
HbA1c (%)	3.59 ± 0.22^{bc}	2.25 ± 0.30^c	2.22 ± 0.36^c	11.27 ± 1.57^a	9.56 ± 1.30^a	6.06 ± 0.89^b
TC (mg dL^{-1})	196.33 ± 8.60^{bc}	188.71 ± 4.12^{bc}	183.72 ± 6.65^c	254.90 ± 4.35^a	239.82 ± 3.13^a	206.03 ± 5.07^b
TGs (mg dL^{-1})	147.63 ± 20.31^{ab}	119.84 ± 4.21^b	115.20 ± 5.08^b	172.03 ± 4.47^a	160.61 ± 14.58^a	137.90 ± 0.93^{ab}
LDL-C (mg dL^{-1})	119.06 ± 11.43^b	105.85 ± 2.27^b	102.64 ± 6.62^b	197.41 ± 6.81^a	175.56 ± 2.65^a	119.27 ± 8.57^b
HDL-C (mg dL^{-1})	47.73 ± 1.52^b	58.89 ± 4.11^a	65.65 ± 1.13^a	23.09 ± 1.67^d	32.14 ± 1.49^c	59.19 ± 3.94^a
VLDL-C (mg dL^{-1})	29.53 ± 4.15^{ab}	23.97 ± 0.84^b	23.04 ± 1.02^b	34.41 ± 0.89^a	32.12 ± 2.91^a	27.58 ± 0.18^{ab}
TGs/HDL-C ratio	3.09 ± 0.43^c	2.05 ± 0.13^d	1.75 ± 0.05^d	7.51 ± 0.39^a	4.98 ± 0.23^b	2.34 ± 0.15^{cd}
TC/HDL-C ratio	4.13 ± 0.31^c	3.23 ± 0.18^c	2.79 ± 0.08^c	11.19 ± 1.05^a	7.49 ± 0.29^b	3.51 ± 0.29^c
AI	0.48 ± 0.15^c	0.31 ± 0.03^d	0.27 ± 0.02^d	0.88 ± 0.02^a	0.70 ± 0.02^b	0.37 ± 0.03^d
MDA ($\text{nmol g}^{-1} \text{ tissue}$)	14.70 ± 1.53^c	14.46 ± 1.43^c	10.73 ± 1.70^d	27.65 ± 0.59^a	24.28 ± 2.00^a	19.75 ± 1.01^b

Values are Means $\pm$ SEM, Values with different small superscript letters in the same row are significant at $p > 0.05$, FBG: Fasting blood glucose, TC: Total cholesterol, TGs: Triglycerides, HbA1c: Hemoglobin A1c, LDLC: Low density lipoprotein cholesterol, HDLC: High density lipoprotein cholesterol, VLDLC: Very low density lipoprotein cholesterol, AI: Atherogenic index, MDA: Malondialdehyde

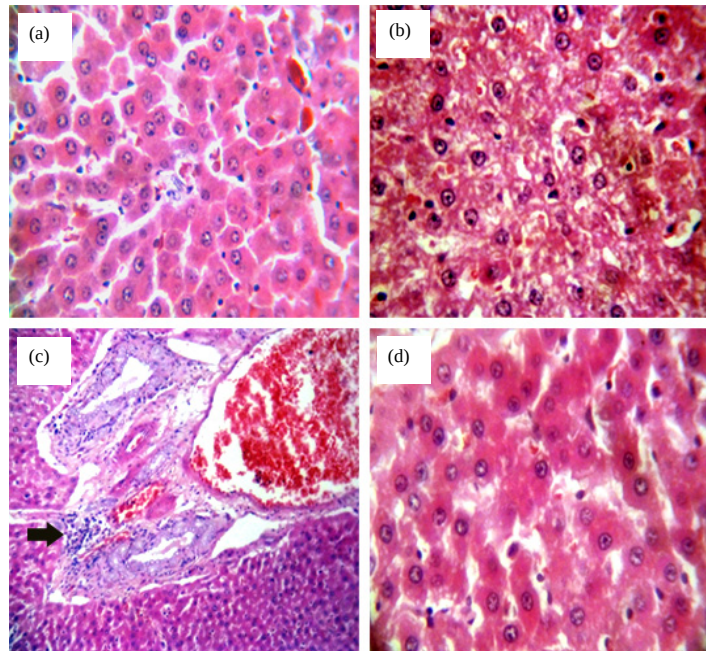


Fig. 1(a-d): Liver of rats showing: (a) Normal histological structure of hepatocytes (group 1, HE: 200), (b) Marked hepatocytes degeneration (group 4, HE: 200), (c) Congestion and MNCs infiltration in portal area (arrow) (group 5, HE: 100) and (d) Normal hepatocytes (group 6, HE: 200)

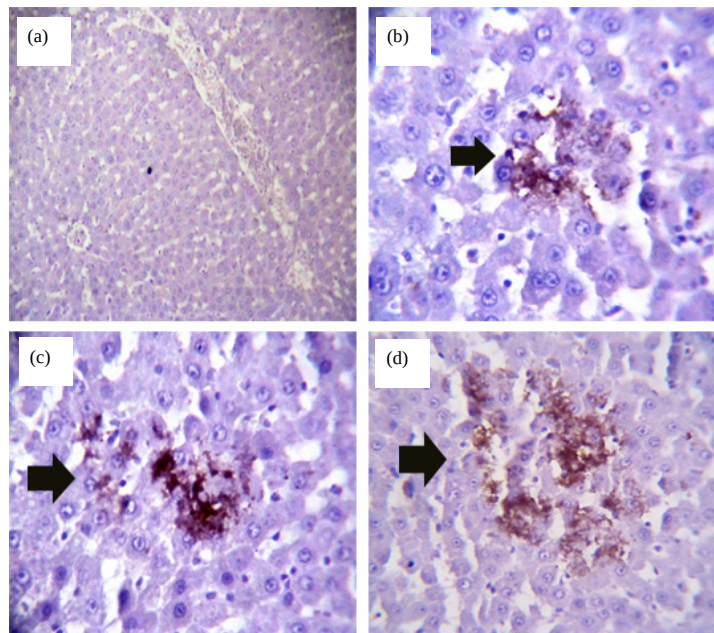


Fig. 2(a-d): Immunohistochemistry labelling of caspase-3 in liver: (a) Negative (100×) and b, c and (d) Arrows point to positive immunolabeling score 1, 2 and 3, respectively (200×)

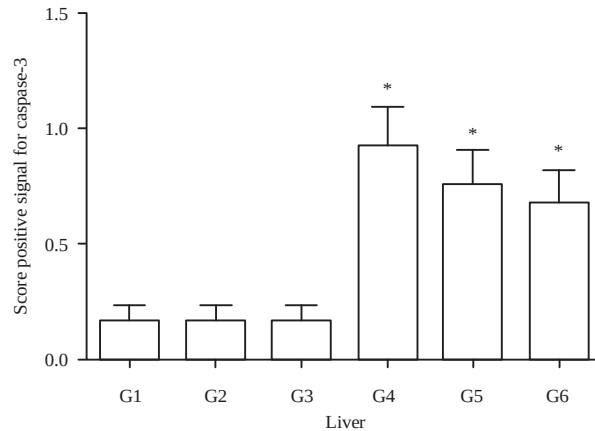


Fig. 3: Immunohistochemistry labelling of caspase-3 in liver. Significance is at ($p < 0.05$)

with no visible lesion. However, pancreas of diabetic rats in group 4 revealed marked degeneration, shrinkage, necrosis and fibrosis of the Islet of Langerhans with severe cytoplasmic degranulation and vacuolization of the exocrine tissue. Meanwhile, in diabetic rats received low and high dose of WGO, the exocrine and endocrine components of pancreas retained normal histology (Fig. 4a-d). Strong immunoreactions of caspase-3 were observed in pancreatic islets from diabetic rats in group 4 that were higher than in other groups (Fig. 5a,b and 6).

DISCUSSION

The current study investigated whether WGO had a protective role against harmful metabolic effects of DM or not through evaluation of biochemical and hormonal assay and histopathological examination of liver and pancreas. Persistent hyperglycemia produced by DM increases the production of free radicals especially ROS as superoxide that generated from glucose auto-oxidation and associated with formation of glycated proteins through polyol pathway, prostanoid synthesis and protein glycosylation (Kumawat *et al.*, 2009). Oxidative stress increased in DM due to the increased production of oxygen free radicals and deficiency in antioxidant defense mechanisms (Rodino-Janeiro *et al.*, 2010). The MDA is the lipid peroxidative marker that increased in STZ induced diabetic rats as reported by Firoozrai *et al.* (2007), Budin *et al.* (2009), Samarghandian *et al.* (2013) and Kumar *et al.* (2013a) found that the oxidative stress in STZ induced diabetic rats was indicated by decreased levels of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and the continuous oxidative stress caused the LPO that was indicated by increased level of MDA. In addition, the increased glucose level in STZ induced diabetic rats causing glucose binding to the N terminal of hemoglobin chain and producing HbA1c that increased up to 16% (Kumar *et al.*, 2013b). The HbA1c was used as an indicator of metabolic control of diabetes (Kumar *et al.*, 2013a). The risk of drug tolerance is high with oral hypoglycemic agents, thereby causing a raise in dosage or change the drug (Srivastava *et al.*, 2012). Several plants have been accounted as an anti-diabetic effect by a variety of mechanisms such as stimulating the regeneration of islets of Langerhans in pancreas, improving insulin sensitivity and augmenting glucose dependent insulin secretion in STZ induced diabetic rats (Daisy *et al.*, 2009). The WGO is the nutritional oil derived from expeller-pressed wheat germ that contains lipids and lipid soluble vitamins (Alessandri *et al.*, 2006). The WGO

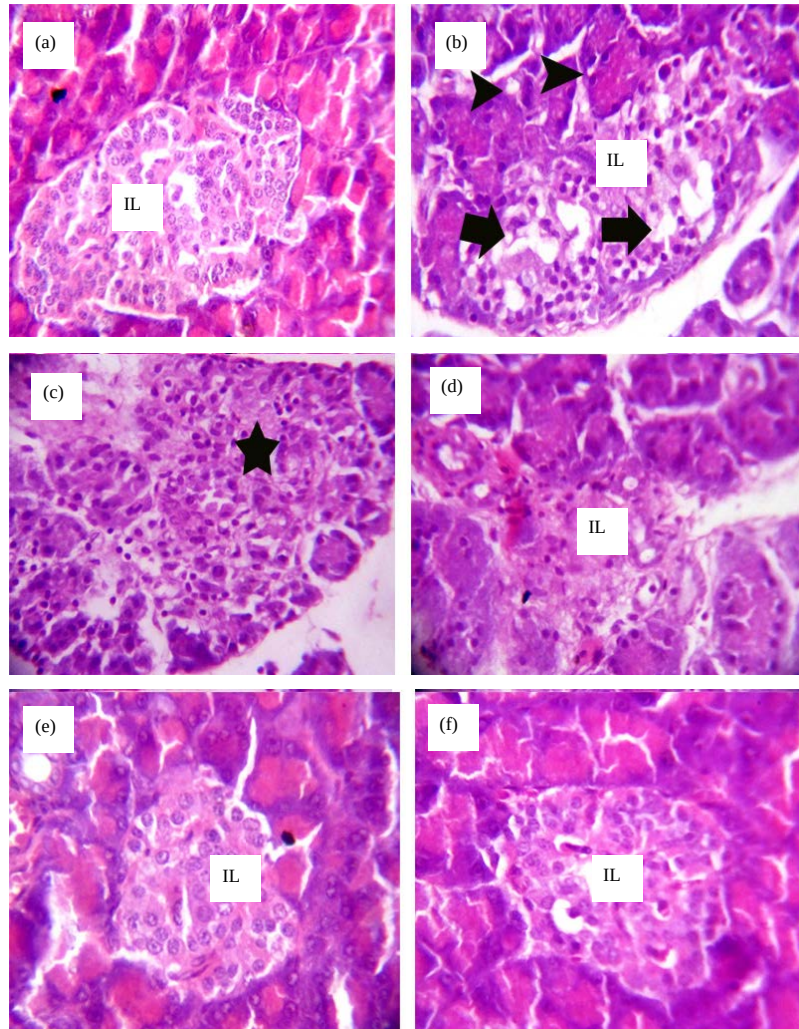


Fig. 4(a-f): Histopathological examination of pancreas revealed (a) Revealed normal appearance of the IL: Islets of Langerhans and the exocrine tissue (group 1, HE×150), (b) Severe cytoplasmic vacuolization of IL (arrows) and the exocrine tissue (arrowheads) (group 4, HE×200), (c) Severe necrosis in the exocrine tissue (asterisk) (group 4, HE×150), (d) Fibrosis of IL (group 4, HE×200), (e, f) Normal appearance of IL and the exocrine tissue (groups 5 and 6, respectively, HE×200).

makes up only 7-12% of the wheat kernel is the richest known source of natural vitamin E (powerful antioxidant) and tocopherols (Zhu *et al.*, 2011).

Oral administration of STZ induced diabetic rats with high dose of WGO (500 mg kg⁻¹ b.wt.) significantly ($p < 0.05$) ameliorated the adverse metabolic effects of DM as it significantly lowered levels of FBG, MDA and HbA1c and significantly ($p < 0.05$) increased insulin levels diabetic rats. In this study, STZ induced diabetic rats had hyperlipidemia with significant elevation of TC, TGs, LDL-C, VLDL-C and AI and significantly decreased HDL-C. Kumar *et al.* (2013a) reported that levels of serum lipids elevated 2 times more in STZ induced diabetic rats when compared to normal

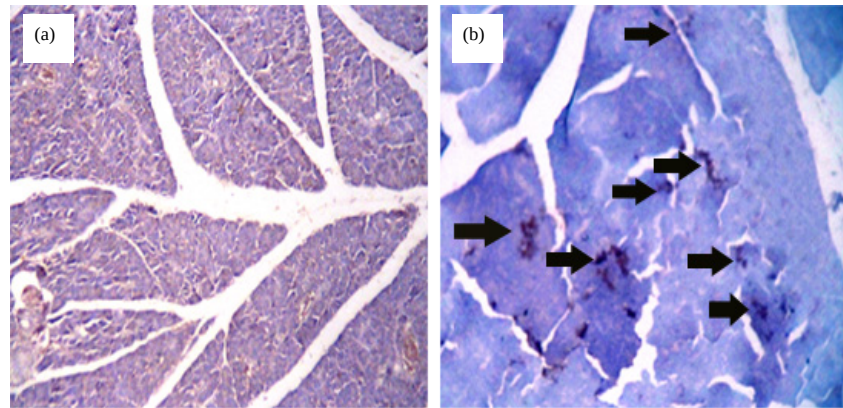


Fig. 5(a-b):Immunohistochemistry labelling of caspase-3 in pancreas: (a) Negative (50×) and (b) Arrows point to positive immunolabeling (100×)

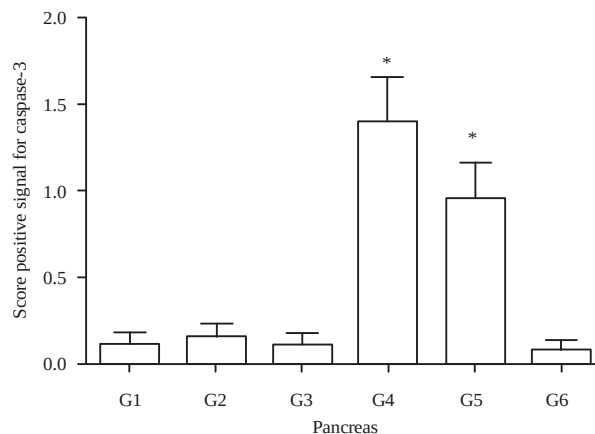


Fig. 6: Immunohistochemistry labelling of caspase-3 in pancreas. Significance is at ($p < 0.05$)

control rats. The hypercholesterolemia and hypertriglyceridemia are mostly found in DM due to lipid abnormalities (Shepherd, 2005). The level of TGs increased due to insulin deficiency result in failure to activate lipoprotein lipase thereby causing hypertriglyceridemia (Shirwaikar *et al.*, 2005). In this study, the oral administration of high dose of WGO induced a significant improvement ($p < 0.05$) in lipid profile of both control negative rats and STZ induced diabetic rats. The possibility of anti-diabetic effect of WGO may be due to the effect of active constituents, namely vitamin E (tocopherols). The latter is a strong antioxidant, lipophilic and inhibits LPO which occurs in plasma membrane and damages the membrane structure and permeability through scavenging lipid peroxy radicals to yield lipid peroxides and the tocoperoxy radicals and improving beta cells function. The latter might influence protein glycation, lipid peroxidation, insulin secretion/sensitivity and it may also affect non oxidative glucose metabolism (Srivastava *et al.*, 2012). In addition to tocopherols, WGO contains also alpha and gamma tocotrienols (Hassanein and Abedel-Razek, 2009). The tocotrienols not only reduced serum glucose and glycated hemoglobin, plasma TC, TGs, LDL-C, MDA and oxidative nucleic acid damage but also increased

HDL-C, increased superoxide dismutase antioxidant enzyme. Thus lowering blood glucose level and oxidative stress marker improves dyslipidemia and maintains vessel wall integrity (Budin *et al.*, 2009). Wheat germ oil also contains policosanols; a substance that can be helpful in lowering raised blood sugar and/or cholesterol levels (Irmak and Dunford, 2005). Antioxidants such as flavonoids, polyphenols, vitamin C and E and carotenoids have been reported to protect the body system against ROS (Mohamed *et al.*, 2010).

Moreover, chronic hyperglycemia in DM is associated with long term damage, dysfunction and failure of various organs. Liver is an insulin dependent tissue that plays a bio-vital role in glucose and lipid homeostasis and severely affected by DM (Lyra *et al.*, 2006). In addition, STZ damages β cells of the islets of Langerhans in the pancreas (Zheng *et al.*, 2015). The destruction of pancreatic β -cells in the islets of Langerhans and loss of insulin secretion are caused by immune-mediated recognition of islet β -cells by auto-reactive T cells and subsequently the liberation of pro-inflammatory cytokines and ROS (Delmastro and Piganelli, 2011; Kaneto *et al.*, 2007). Cell death is the last stage of the cellular damage and it can occur by apoptosis or necrosis. These data supported our histopathological findings in liver and pancreas of untreated diabetic rats. In addition, our results showed that, high dose of WGO was effective in relieving histopathological lesions caused by DM. Caspase-3 is the main marker of the apoptosis (Slauson and Cooper, 2002). Caspases are cysteine-aspartyl specific proteases that play a key role in apoptosis (Creagh *et al.*, 2003). Caspase-3 is one of the effector caspases downstream of apoptotic pathways. Several *in vitro* studies have suggested that caspase dependent apoptotic pathways are essential for β -cell apoptosis (Maedler *et al.*, 2001). Strong immunoreactions of caspase-3 were observed in liver and pancreatic islets of untreated diabetic rats that were higher than treated and non diabetic groups. The increased apoptosis can cause marked lesions in organs. Thus, the ability of the high dose of WGO to significantly decrease the apoptosis can protect the organs from harmful effects of DM.

CONCLUSION

The present investigation indicated that a high dose of WGO (500 mg kg⁻¹ b.wt.) has anti-diabetic, anti-hyperlipidemic and antioxidant efficacy in DM disease, so it has a protective effect against diabetic complications.

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