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ABSTRACT

Thiswork aimed to study protective effects of oral administration of wheat germ oil (WGO 500 mg/kg B.wt) on hyperlipidemia and brain oxidative stress in hypercholesterolemic (HC) and streptozotocin (STZ) -diabetic rats. Five groups of male Sprague Dawely rats were used; group 1: control negative, group 2: HC rats, group 3: STZ- diabetic rats, group 4: HC rats treated with 500mg/kg B.wt WGO and group 5: STZ-diabetic rats treated with 500mg/kg B.wt WGO. After 6 weeks (the end of the experimental period), serum of all rat groups were used for lipid profile analysis to determine the levels of total cholesterol (TC), triacylglycerol (TAG), 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. Brain tissue homogenates from all rat groups, were used for evaluation of malondialdehyde (MDA) level, antioxidant enzymes activities like superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). Specimens from cerebrum were microscopically examined. HC rats (G2) and STZ-diabetic rats (G3) showed a significant elevation of serum lipid profile, atherogenic index (AI) and brain MDA level, while there was a significant decrease in serum HDL-C and antioxidant enzymes activities in brain tissue homogenate when compared with control negative group (G1). Oral administration of 500mg/kg B.wt WGO to HC and STZ-diabetic rats, significantly ($p < 0.05$) reduced serum lipid profile, AI and brain MDA, however significantly ($p < 0.05$) increased serum HDL-C levels and antioxidant enzymes activities in brain tissue homogenate when compared with untreated HC and STZ- diabetic rats. Histopathological examination of cerebral tissue revealed that WGO treatment of HC and STZ-diabetic rats showed normal histology.

Abbreviations: *WGO, wheat germ oil; B.wt, body weight; TC, Total cholesterol; STZ, streptozotocin. TGs, Triglycerides; LDL-C, Low density lipoprotein- cholesterol; VLDL-C, Very low density lipoprotein- cholesterol; HDL-C, High density lipoprotein-cholesterol; AI, Atherogenic Index ; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; PBS, phosphate buffer saline; H&E, hematoxylin and eosin.*

INTRODUCTION

Hyperlipidemia is considered as the primary mediator of a cascade of heart damaging events in addition to stroke and atherosclerosis **(Balakumar et al., 2007)**, pancreatitis and renal injury **(Attia et al., 2002)** and metabolic syndrome **(Xu et al., 2007).** It may be due to hereditary disorders (Familial hypercholesterolemia), polygenic hypercholesterolemia (Familial combined hypercholesterolemia) or non-lipid diseases such as type 2 diabetes, nephritic syndrome, hypothyroidism and obesity **(Frederick, 2009). Poli et al., (2008)** demonstrated that hypercholesterolemia is associated with elevated plasma TAGs and/or reduced HDL-C. An abnormal ratio of TAGs to HDL-C indicated an atherogenic lipid profile **(Da Luz et al., 2008)**. Cardiovascular disease including

coronary heart disease and stroke are the cause of mortality in both developed and developing countries, accounting for roughly 20% of all worldwide deaths per year **(Thomas and Rich, 2007)**. Hypercholesterolemia induces oxidative stress by causing a reduction in the enzymatic antioxidant defense potential of tissues, generation of free radicals and an imbalance between free radicals production and antioxidant levels leading to elevated oxidative stress and accelerated lipid peroxidation, cellular injury, atherosclerosis and heart diseases **(Shah et al., 2007).**

Diabetes mellitus considered as one of the most common chronic diseases worldwide and recognized as one of the leading causes of morbidity and mortality **(American Association of Diabetes Educators, 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 diabetes mellitus in experimental animals **(Shrilatha and Muralidhara, 2007).** 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 **(Kumar et al., 2011)**. Several studies have shown that cytotoxic effects of STZ were produced by the releasing of reactive oxygen species the intracellular liberation of nitric oxide **(Szkudelski, 2001)** and the imbalance between plasma oxidant and antioxidant content, DNA alkylation **(Elsner et al., 2000).**

There is growing scientific evidence of the protective biochemical functions of naturally occurring antioxidants in biological systems **(Ashour et al., 2011).** Antioxidants such as flavonoids, polyphenols, vitamin C and E and carotenoids have been reported to protect the body system against reactive oxygen species (**Mohamed et al., 2010).** Wheat germ oil (WGO), known to be the richest natural source in tocopherols, contains all three alpha, beta and gamma tocopherols, alpha and gamma-tocotrienols, and also it contains phytosterols, mainly camp sterol, beta-cytosterol, and to a less extent 5- stigma sterol, 7- stigma sterol, isofucosterol and 7-avenasterol, these compounds have strong antioxidant activity **(Hassanein and abdel-Razek, 2009). Alhassan et al., (2006)** reported that the plant sterols inhibit cholesterol absorption, lower serum cholesterol, confer a healthier lipid profile and ameliorate cardiovascular disease risk factors **(Ziv et al., 2009).**

MATERIALS AND METHODS

1- MATERIALS:

1.a- Experimental animals:

The study was conducted on thirty mature *Sprague Dawely* male rats weighting 130- 160 g body weight. Animals were purchased from the animal house in Helwan- Egypt and were housed in separate cages. All animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Egypt. Registration number (09/189).

1.b- Diet and additives:

Normal control ration:

Normal control ration constituents were obtained from Faculty of Agriculture, Mansoura University. The diet was pelleted composed of (Ground yellow corn, Wheat bran, Soya bean meal, Corn gluten, Limestone, Sodium chloride, Vitamins and Minerals and Molasses). The ingredients of the basal control diet were shown in **(Table 1)** to meet the recommended nutrients requirements for growth of laboratory rats according to **National Research Council (1995).**

Cholesterol rich diet (Hypercholestrolemic diet):

Hypercholestrolemic diet was prepared by formulation of the basal control diet with cholesterol 1%, cholic acid 0.5% and 5% corn oil **(Table 1)** as described by **Zulet et al., (1999).** Cholesterol and cholic acid were purchased from Beta- Scientific office for chemicals- Egypt.

1.c- Wheat germ oil (WGO):

WGO was purchased from Sedico Pharmaceutical Company (Extra- 1000- Sedico)- Egypt 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 500 mg/kg B.wt.

1.d- Chemicals:

Diethyl ether for anesthesia, STZ was used for experimental induction of diabetes type 1 in rats in the form of vial containing 1 gm STZ powder, cold 0.1 M citrate buffer for STZ dissolving, cold phosphate buffer saline (50 Mm potassium phosphate, pH 7.5) for brain tissue homogenate, kits for analysis of serum triacylglycerols (TAGs), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and MDA, SOD, CAT and GSH in brain tissue homogenate.

1.e-Equipments:

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

2-METHODS:

2-a-Induction of experimental diabetes in rats by STZ:

For induction of type 1 DM. Rats were fasted overnight then injected intraperitoneally (I.P) 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 **Mohamed et al., (2013)**. The animals were given 5% glucose water for 24 hours following STZ injection to prevent initial drug induced hypoglycemic mortality. After I.P injection of STZ, the rats were fasted for 14 hours, 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 were considered diabetic and were included in this study. Treatment with WGO was started 3 days after the induction of diabetes and continued for 6 weeks the duration of experiment.

2-b-Experimental design:

Thirty rats were randomly divided into five groups (6x5)**,** group (1) control negative,

groups (2) hypercholesterolemic (HC) rats, group (3) STZ- diabetic rats, group (4) HC rats orally gavage with WGO (500mg/kg B.wt) and group (5) STZ-diabetic rats orally gavage with WGO (500mg/kg B.wt).

2-c-Sampling:

2-c (1)-Blood samples:

At the end of the experimental period, the rats were fasted overnight, and then anaesthetized by using diethyl ether. Blood samples were withdrawn from retro orbital plexus of all rats, immediately collected in a tube left in a vertical position at room temperature then centrifuged at 3000 rpm for 15 minutes. A clear, straw colored serum sample aspirated by automatic pipette and transferred into clean, dry, labeled tubes and kept at -20^{o}C for subsequent biochemical analysis of serum lipid profile.

2-c (2)-Tissue samples:

Rats were dissected at the end of the experimental period. Brain samples were collected from each rat, washed with normal physiological saline to clean the remaining blood. One gram of the brain tissue was used for preparation of brain tissue homogenate. The remaining parts of the cerebral hemispheres were fixed in 10% formalin for histopathological examination.

2-d-Preparation of brain tissue Homogenate:

One gram of brain 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 3ml supernatant was aspirated, collected into separate Eppendorf tubes and stored at -20ºC for further biochemical analysis of MDA, SOD, CAT and GSH.

2-e-Biochemical analysis:

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 triacylglycerol to high density lipoprotein (log[TAG/HDL-C]) according to what previously described by **Dobiasova and Frohlich (2001).** Brain tissue MDA (nmol/g tissue) was assessed by using commercial kit according to **Raquel et al., (2005).** Brain tissue SOD activity (U/g tissue) was assessed by using commercial kit according to **Nishikimi et al., (1972)**. Brain tissue CAT activity (U/g tissue) was assessed by using commercial kit according to **Aebi, (1984)** and brain tissue GSH level (mg/g tissue) was assessed by using commercial kit according to **Beutler et al., (1963).**

2-f-Histopathological examination:

Fixed specimens were processed routinely until embedding in paraffin wax. Paraffin sections of 5µm thickness were cut and picked up on uncoated slides, dried, deparafinized, rehydrated with graded alcohol, washed and stained with H&E according to **Bancroft et al., (1996).** Histological changes were examined by light microscopy (binocular, Olympus). Images were taken using Digital camera (Canon 5 mega pixels, 3.2x optical zoom).

2-2-g-Statistical analysis:

All the data of the animal experiments were expressed as means ±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. Results were considered significant only at the level of (P ‧ 0.05) or less.

RESULTS

1-Effects of WGO (500mg/kg B.wt) on serum lipid profile:

Significant ($p<0.05$) increases in serum levels of TC, TAGs, LDL-C, VLDL-C, ratios of TC/HDL-C and TAGs/HDL-C, AI and significant ($p<0.05$) decreases in HDL-C were shown in HC rats and STZ- diabetic rats when compared to control rats. The treatment of HC rats and STZ-diabetic rats with WGO 500mg/kg B.wt induced a significant improvements in the serum lipid profile**,** decreases serum levels of TC, TAGs, LDL-C, VLDL-C, ratios of TC/HDL-C and TAGs/HDL-C, AI and significantly ($p<0.05$) increases HDL-C levels when compared to untreated groups **(Table 2)**.

2- Effects of WGO on MDA and antioxidant enzymes activities in brain tissue homogenate in different experimental groups:

A significant (P‧ 0.05) increases in MDA levels (25.14±2.24 nmol/g tissue) and a significantly (P‧ 0.05) decreased activities of antioxidant enzymes, SOD activity was (2.37±0.57 U/g tissue), CAT activity was (0.21± 0.12 U/g tissue) and GSH activity was (3.81± 0.32 mg/g tissue) were recorded in HC rats when compared to those levels obtained for control rats. Similarly for the diabetic rats, a significant (P‧ 0.05) increases in MDA levels (15.31±2.64 nmol/g tissue) and a significantly (P‧ 0.05) decreased antioxidant enzymes (SOD, CAT and GSH) were reported (8.71± 1.88 nmol/g tissue). Treatment of HC rats and diabetic rats with WGO 500 mg/kg B.wt significantly reduced oxidative stress in brain tissues of rats as it significantly (P‧ 0.05) decreased MDA levels and significantly (P‧ 0.05) increased activites of antioxidant enzymes when compared to those levels obtained for untreated groups **(Table 3)**.

3- Histopathological finding of brain tissue in different experimental groups:

Histopathological finding of cerebral sections of control rats showed normal histopathological picture **(Fig.1A)**. Meanwhile, brain sections of HC rats showed neuronal degeneration with prominent satellitosis, diffuse gliosis, particularly, around damaged blood vessels with few extravasated erythrocytes **(Fig.1B&C)**. Brain sections of diabetic rats showed perivascular gliosis around congested blood vessels **(Fig.1D&E)** and focal perivascular hemorrhage**.** The microscopic pictures of brain sections of treated HC and diabetic rats with 500mg/kg B.wt WGO showed normal histopathological appearance **(Fig. 1F)**.

Table (1): Composition of the control and HC diets (g/ 100g).

Ingredients	Control diet	Hypercholesterolemic diet
Ground yellow corn	71.2%	62.7%
Wheat bran	7%	9%
Soya bean meal	5%	2.5%
Corn gluten	9%	11.5%
Lime stone	2%	2%
Di-Calcium phosphate	2%	2%
Sodium chloride	0.5%	0.5%
Premix	0.3%	0.3%
Molasses	3%	3%
Cholesterol	0	1%
Cholic acid	0	0.5%
Corn oil	0	5%

Table (2): Effects of WGO on serum lipid profile

Parameters	Experimental groups				
	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)
TC (mg/dl)	196.33±8.60^{bc}	338.98±22.75^{a}	254.90±4.35^{a}	213.65±1.89^{bc}	206.03±5.07^{b}
TGs (mg/dl)	147.63±20.31^{bc}	230.97±1.46^{a}	172.03±4.47^{a}	148.44±0.88^{bc}	137.90±0.93^{ab}
LDL-C (mg/dl)	119.06±11.43^{b}	246.72±22.86^{a}	197.41±6.81^{a}	122.33±2.93^{b}	119.27±8.57^{b}
HDL-C (mg/dl)	47.73±1.52^{b}	46.07±1.39^{b}	23.09±1.67^{d}	61.62±1.01^{a}	59.19±3.94^{a}
VLDL-C (mg/dl)	29.53±4.15^{bc}	46.19±0.29^{a}	34.41±0.89^{a}	29.69±0.18^{bc}	27.58±0.18^{ab}
TGs/HDL-C ratio	3.09±0.43^{b}	5.02±0.15^{a}	7.51±0.39^{a}	2.38±0.01^{bcd}	2.34±0.15^{cd}
TC/HDL-C ratio	4.13±0.31^{b}	7.38±0.57^{a}	11.19±1.05^{a}	3.47±0.09^{bc}	3.51±0.29^{c}
AI	0.48±0.16^{b}	0.70±0.01^{a}	0.88±0.02^{a}	0.38±0.01^{bc}	0.37±0.03^{d}

- Values are means ±S.E.
- Values with different letters in the same row are significant at (P‧ 0.05).

Table (3): Effects of WGO on MDA and antioxidant enzymes levels in brain tissue homogenate

Parameters	Experimental groups				
	Group (1)	**Group (2)**	**Group (3)**	**Group (4)**	**Group (5)**
MDA(nmol/g tissue)	8.71±1.88^{c}	25.14±2.24^{a}	15.31±2.64^{a}	14.58±1.43^{bc}	8.47±0.33^{b}
SOD (U/g tissue)	14.73±2.25^{b}	3.37±0.57^{c}	1.61±0.25^{d}	13.41±1.25^{b}	10.25±1.65^{bc}
CAT (U/g tissue)	1.03±0.08^{ab}	0.21±0.12^{d}	0.25±0.12^{c}	0.64±0.02^{bc}	0.71±0.19^{bc}
GSH (mg/g tissue)	7.82±1.13^{ab}	3.81±0.32^{c}	4.01±0.85^{b}	7.48±0.21^{ab}	8.77±0.44^{a}

Values are mean ±SE.

Values with different letters in the same row are significant at (P• 0.05).

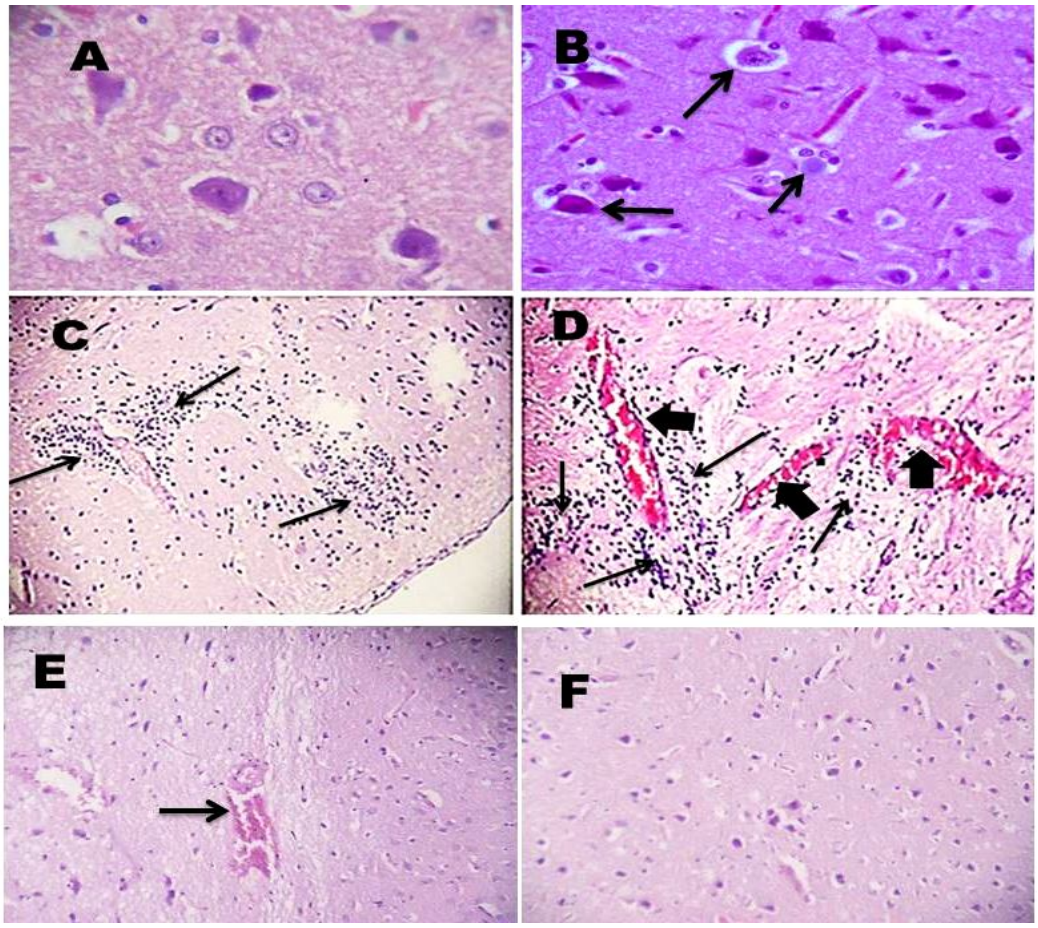

Fig. 1 (A-F): Histopathological finding of brain sections shows **(A):** Normal histological picture in (control group, H&E, X: 200). **(B):** Neuronal degeneration with prominent satellitosis (arrows) in (HC group, H&E, X: 200**)**. **(C):** Diffuse gliosis (arrows) in (HC group, H&E, X: 100**)**. **(D):** Perivascular gliosis (thin arrows) around congested blood vessels (thick arrows) in (diabetic group, H&E, X: 200**)**. **(E):** Focal perivascular hemorrhage (arrow) (diabetic group, H&E, X: 100**)**. **(F):** Normal histological picture in (treated groups, H&E, X: 100).

DISCUSSION

Antioxidants such as flavonoids, polyphenols, vitamin C and E and carotenoids have been reported to protect the body system against reactive oxygen species (**Mohamed et al., 2010).** The present study aimed to evaluate the effect of oral administration of WGO 500 mg/kg B.wt of rat as a natural antioxidant (ameliorating agent) on serum lipid profile, lipid peroxidation and antioxidant enzymes in brain tissues of HC and STZ-diabetic rats. Hypercholesterolemia, high-cholesterol diet, elevated serum TC and LDL-C levels and oxidative stress resulting in increased risk for atherosclerosis development **(Hakimoglu et al., 2007).** Cell membranes contain unsaturated fatty acids and due to this property are a target for free radicals that resulted in peroxidation of lipid membranes and generation of MDA **(Migliore and Coppede, 2009).** Also hypercholesterolemia is the major cause for ischemic brain damage (ischemic stroke), that caused by occlusion of a major cerebral artery, resulted in significant reduction in regional cerebral blood flow, causes deprivation of oxygen and glucose and resulted in brain damage **(Hai et al., 2011)**. There is a link between exposure to high dietary fat and/or cholesterols and oxidative stress in brains of mice and rats that reflected by an up regulation of reactive oxygen species production, oxidized nucleotides and proteins and markers of lipid peroxidation **(Zhang et al., 2005)**.

In this study HC rats demonstrated a significant elevation of serum TC, TAGs, LDL-C, VLDL-C, TC/HDL-C, TAGs/HDL-C, AI and a significant reduction in the serum HDL-C as compared with control negative group fed cholesterol-free, this result in agreement with that reported by **(Yang et al., 2008; Ma et al., 2011)**. Feeding rats a diet rich in cholesterol evoked an increase in risk factors for atherosclerosis and cardiovascular disease such as dyslipidemia (high TAG, TC, LDL-C, and low HDL-C) and lipid peroxidation (increased MDA levels). This general profile elicited by an unhealthy dietary pattern (atherogenic diet) **(Mohamed and Doha, 2009)**. Also results obtained from the present study revealed the elevation of oxidative stress in the brain of HC rats which was reflected by a reduction in the levels of antioxidant enzymes including superoxide dismutase, catalase and reduced glutathione and a by elevation of MDA levels, which is the indicator of lipid peroxidation that induced by free radicals damaging the cell membrane. These results are similar to that reported by **Zhang et al. (2005) and Chen et al., (2009)** who reported that cerebral ischemia can cause a significant amount of MDA formation in ischemic hemisphere. Also it was reported by **Montilla et al., (2006)** that a high cholesterol diet reduced activity of several antioxidant enzymes such as catalase and superoxide dismutase in rat's brain. An imbalance between free radicals production and antioxidant levels leads to oxidative stress in the brain **(Mena et al., 2009)**. Also cholesterol may initiate amyloid- *ß* (*Aß)* formation, which mentioned as a neuron toxic, a potent source of oxidative stress and irreversible protein aggregation **(Betul and Nesrin, 2011)**.

Treatments of HC rats by oral administration of WGO 500 mg/kg B.wt produced an improvement in the serum lipid profile of rats and ameliorated brain oxidative stress and improved the microscopic picture of brain as the therapeutics targeting hypercholesterolemia decreases the risk of ischemic stroke in high risk individuals or in patients with transient ischemic attack **(Amareco and Labreuche, 2009)**.

STZ induced diabetic rats had hyperlipidemia with significant elevation of TC, TAGs, LDL-C, VLDL-C and AI and significantly decreased HDL-C. **Kumar et al., (2013)** reported that levels of serum lipids elevated 2 times more in STZ induced diabetic rats when compared to normal control rats. The hypercholesterolemia and hypertriglyceridemia are mostly found in diabetes mellitus due to lipid abnormalities **(Shepherd**, **2005**). The level of TAGs increased due to insulin deficiency result in failure to activate lipoprotein lipase thereby causing hypertriglyceridemia **(Shirwaikar et al., 2005)**. Significant increase in MDA levels and significant decrease in antioxidant enzymes activities in brain tissue revealed that DM induced brain oxidative stress as mentioned by **Maritim et al. (2003) and Mohammadi et al. (2013)**. Hence, histopathological lesions were detected only in HC and diabetic rats including neuronal degeneration with prominent satellitosis, gliosis, congestion and focal perivascular hemorrhage**.** Neuronal degeneration has been reported to occur in diabetic retinopathy (**Lecleire-Collet et al., 2005**) which may be due to microvascular abnormalities.

The oral administration of high dose of WGO induced a significant improvement ($p<0.05$) in lipid profile in STZ induced diabetic rats and reduced brain oxidative stress biomarker, MDA and increased antioxidant enzymes activities. Histopathological finding of brain sections from treated rats came to confirm the biochemical results where normal histological picture was shown in treated groups. This result may be attributed to vitamin E content of WGO that gives a powerful antioxidant protection to any organ **(Field et al., 2008)**.

In addition to tocopherols that are the abundantly found structural components of WGO **(Liu et al., 2008)**, carotenoids, phenolic compounds **(Zhu et al., 2011)**, essential fatty acids **(Hassanein and Abdel-Razek, 2009)** and certain phytosterols **(Alessandri et al., 2011)** were also found in the structure of WGO and all have antioxidant effects.

CONCLUSION

The present investigation indicated that WGO (500 mg/kg B.wt) has a protective effect against hypercholesterolemia and diabetes-induced hyperlipidemia and brain oxidative stress, it produced improvements in biochemical assays and relieved the histopathological changes in HC and diabetic rats.

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الملخص العربي

تأثير الحماية الناشئة من زيت جنين القمح لمواجهة نقص تدفق الدم إلي المخ وإجهاد الآكسدة في الجرزان المصابة بإرتفاع نسبة الكوليسترول في الدم والآخرى المصابة بمرض السكري

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تهدف هذة الدراسة الى تقييم مدى تأثير زيت جنين القمح بجرعه ٥٠٠ مجم/كجم علي ارتفاع نسبة دهون في الدم وعلي حدوث اجهاد التأكسد لنسيج المخ لمجموعتي من الجزران، الآولي مصابة بإرتفاع نسبة الدهون في الدم ، والثانية مصابة بمرض السكر . إستمرت هذة الدراسة لمدة ستة أسابيع و أجريت على عدد ثلاثون من الجرزان، تم تقسيمهم إلى ستة مجموعات إشتملت كل مجموعة على عدد خمس جرزان وكان توزيع المجموعات كالتالى: المجموعة الآولى (المجموعة الضابطة) المجموعة الثانية (مجموعة الجرزان المصابة بإرتفاع نسبة الكوليسترول في الدم والتى تتغذي علي عليق غنية بالكوليسترول) المجموعة الثالثة (مجموعة الجرزان المصابة بمرض السكر الذي تم إحداثة عن طريق حقن مادة الإستربتوزوتوسين بجرعة ٥٠ مجم/كجم من وزن الحيوان فى الغشاء البريتونى) المجموعة الرابعة (مجموعة الجرزان المصابة بإرتفاع نسبة الكوليسترول في الدم والتي تتلقى جرعة يومية (٥٠٠مجم/ كجم من وزن الحيوان) من زيت جنين القمح بواسطة أنبوبة المعدة المجموعة الخامسة (مجموعة الجرزان المصابة بمرض السكر والتي تتلقى جرعة يومية (٥٠٠مجم/ كجم من وزن الحيوان) من زيت جنين القمح بواسطة أنبوبة المعدة . تم تحليل صورة الدهون في مصل الدم للمجموعات المختلفة ، وأيضا تم أخذ واحد جم من نسيج المخ وتم وضعهم في محلول متعادل لقياس تركيز المالون داي ألدهيد، نشاط إنزيم السوبر أكسيد ديسميوتاز، نشاط إنزيم الكتالاز وأيضا معدل إنزيم الجلوتاثيون المختزل. ثم تم تثبيت الجزء المتبقي من نسيج المخ في محلول متعادل الفورمالين تركيز ١٠% وذلك لإخضاعها للفحص الهستوباثولوجي. تم تحليل النتائج إحصائيا وقد أسفرت النتائج عن أن : نتيجة العلاج بالزيت أدت إلي وجود تحسين ملحوظ فى صورة الدهون ، وأيضا تقليل إجهاد التأكسد فى نسيج المخ والذى يتضح من وجود نقص معنوى فى مستوى المالون داى الدهيد (اكسيد الدهون) مع زيادة معنوية فى نشاط الإنزيمات المضادة للآكسدة فى المجموعة رقم (٥،٤) مقارنة بمتوسط النتائج التى تم الحصول عليها للمجموعتين (٢، ٣) مع نقص ملحوظ فى التغيرات المرضية لنسيج المخ والتى وجدت فى مجموعتي رقم (٣،٢) التى لم تعالج بزيت جنين القمح