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The Antihyperglycaemic Effect of the Aqueous Extract of *Origanium vulgare* Leaves in Streptozotocin-Induced Diabetic Rats

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Abstract

The current study aimed to investigate the antihyperglycaemic effect of aqueous extract of *Origanum vulgare* (oregano, OV) leaves in streptozotocin (STZ) induced diabetic rat. Streptozotocin induced diabetic rats showed significant ($P < 0.05$) increase in the levels of blood glucose, glycosylated haemoglobin, pancreatic amylase, liver and kidney weights /body weight ratios, urea, uric acid, creatinine and significant decrease in the levels of plasma insulin, liver and muscle glycogen and body weight. Oral administration of the aqueous extract of OV leaves (20 mg/kg) produced a significant decrease in blood-glucose levels, glycosylated haemoglobin, pancreatic amylase in STZ diabetic rats ($P < 0.05$) in comparison with standard drug Glibenclamide (GB) (0.9 mg/kg body weight). Treatment with the aqueous extracts of OV leaves decreased liver weights/body weight ratios in diabetic rats, while kidney weight/body weight ratios, urea, uric acid, creatinine levels were partially improved. Oral administration of the aqueous extract of OV leaves (20 mg/kg) improved the reduction in serum insulin, liver and muscle glycogen contents and body weight in STZ diabetic rats. The data in the present study may support the use of *O. vulgare* plants as traditional remedies for the treatment of diabetes mellitus.

Keywords:*Origanum vulgare*, streptozotocin, glycosylated haemoglobin, glucose,insulin, rats**.**

1. Introduction

Diabetes mellitus is a chronic metabolic disease which now afflicts 10 % of the world population. It is considered a "modern-day epidemic" and is rightly recognized as a global public health issue (Gispen and Biessels, 2000; Burke *et al*., 2003). In recent years, there has been renewed interest in the treatment of diabetes using herbal drugs, which are generally non-toxic. World Health Organization has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs. Plant derivatives with hypoglycaemic properties had been used in folk medicine and traditional healing systems around the world from very ancient times (Yeh *et al*., 2003).

Origanum vulgare (oregano, OV) is a member of the plant family Lamiaceae, the genus *Origanum* is an annual, perennial and shrubby herb that is native to the Mediterranean, Euro-Siberian and Irano-Siberian regions (Aligiannis *et al*., 2001). Oregano contains oleanolic and ursolic acids, flavonoids and hydroquinones, caffeic, rosemarinic, and lithospermic acid, tannins, and phenolic glycosides. Phenolic compounds represent 71% of the total oil. The polar phenols' thymol and carvacrol are responsible for many of the properties of the essential oil, as well as p-cymene and terpinene (Dadalioglu and Evredlik, 2004; Giordani *et al*., 2004; Koukoulist *et al*., 2005; Tampieri *et al*., 2005; Bozin *et al*., 2006). The main known pharmacological activities of OV were antibacterial (Nazia *et al*., 2007) antifungal (Portillo-Ruiz *et al*., 2005) antiparasitic (Force *et al*., 2000) anti-thrombin (Goun *et al*., 2002) anti-oxidant (Stashenko *et al*., 2002) and anti-inflammatory (Ocaña-Fuentes *et al*., 2010). There are also some reports regarding the antimutagenic and anticarcinogenic effect of oregano; representing an alternative for the potential treatment and/or prevention of certain chronic ailments, like cancer (Arcila-Lozano *et al*., 2004).

Oregano is a powerful antioxidant. It contains several potent antioxidants that may contribute to the findings in preliminary studies that oregano exhibits benefits towards the cardiovascular and nervous systems and relieves inflammation and modulates blood sugar and lipids (Singletary, 2010).

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The aim of this study was directed to determine the antihyperglycaemic effect of OV extract in streptozotocin diabetic rats. Glibenclamide (GB) was used as standard antihyperglycaemic drug.

2. Materials and Methods

Streptozotocin was purchased from Sigma (St. Louis, MO, U.S.A.) , glibenclamide (gift from Pharmacy, University hospital, Jeddah, Saudi Arabia). Dried leaves of *O.vulgare* were purchased from an authentic source at local market (Jeddah, Saudi Arabia) .

2.1. Preparation of aqueous extracts

Plant material was prepared according to Eddouks *et al*., 2003: 1g of powdered leaves mixed with 100 ml distilled water was boiled for 10 min. and then cooled for 15 min.There after, the aquaeous extract was filtered using a Millipore filter (Millipore 0.2 mm) to remove particulate matter. The filterate was then freeze- dried and the desired dose (mg of lyophilized aquaeous extract of OV leaves per kg body weight) was prepared and reconstituted in 1.5 ml of distilled water. The aquaeous extracts were prepared daily, just before administration. The extracts obtained were then given orally to different groups of rats at a dose of 20 mg /kg body weight.

2.2. Experimental design

The experimental animals were obtained from the Animal Unit of King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia. Animals were housed under environmental conditions (23 ± 1ºC , 55 ± 5% humidity and 12-h light :12- h dark cycle) and maintained with free access to water and a standard chow diet. Diabetes was induced by an intraperitoneal injection of streptozotocin at a dose of 45 mg/kg body weight dissolved in a citrate buffer (0.1M, pH 4.5) (Burcelin *et al*., 1995). After 3 days the rats with fasting blood-glucose levels more than 200 mg/dL were considered as diabetic and selected for the study. All the pharmacological experiments were carried out after obtaining approval of the Institutional animal ethics Committee of King Abdulaziz University, Saudi Arabia.

The preliminary studies of different doses (20,40,60 mg /kg) showed that the most effective dose of oregano is 20 mg /kg. The animals were randomly divided into five groups of 12 animals each. Group I (untreated controls): normal rats receiving water and fed *ad libitum* and served as a control group. Group II (untreated diabetics): diabetic rats receiving water and fed *ad libitum* and served as diabetic control rats. Group III (treated controls): normal rats receiving water and fed *ad libitum* and oregano at 20 mg /kg body weight (Lemhadri *et al*.,2004). Group IV (treated diabetics): diabetic rats receiving water and fed *ad libitum* and oregano 20mg /kg body weight. Group V (treated diabetics):diabetic rats receiving water and fed *ad libitum* and antidiabetic drug (glibenclamide). Rat equivalent dose of glibenclamide was calculated using conversion table devised by Paget and Barnes (1964) and was 0.9 mg/kg body weight.

The drug treatment solutions were administered orally by gastric intubation using a syringe once daily at 08:00 a.m. The effect of OV aqueous extracts or glibenclamide on blood glucose was determined in fasted rats, after 2 and 6 weeks of once daily repeated oral administration (20 mg/kg). The body, liver and kidney weights of all rats were measured at weeks 2 and 6.

2.3. . Biochemical analysis

At the end of experiment, rats were anaesthetized and blood samples were collected from the tail vein. Fasting blood-glucose level was measured in the whole blood after 12 h fasting. Glycosylated haemoglobin (HbA1-test) was estimated in whole blood by fast ion – exchange resin separation method (Nuttall, 1998). Serum was separated, and insulin and pancreatic amylase were determined according to methods of Finlay and Dillard (2007) and Winn-Deen *et al*.(2008), respectively. Liver and muscle glycogen contents were determined by the method of Huijing (1970). Urea, uric acid and creatinine were estimated by using the F-200 fluorescence spectrophotometer in serum (Newman and Price, 2001).

2.4. Statistical analysis

Values reported are expressed as mean ± SE. Statistical significance of the difference between groups was determined by one-way analysis of variance test (ANOVA). The values were considered to be significantly different when the *P* value was less than 0.05 (Zar, 1996).

3. Results

In this study, the level of blood glucose, glycosylated haemoglobin (HbA1C), insulin after using a 45 mg/kg dose of streptozotocin, ensured induction of diabetes in rats (figures 1,2,3). The blood-glucose levels rose markedly after STZ administration, and the high glucose levels were maintained for 6 weeks (Fig 1). Oral administration of the aqueous OV extracts (20 mg/kg) produced a significant decrease (81.65±1.049) in blood-glucose levels in STZ diabetic rats (526.80 ±7.889) ($P < 0.05$). Treatment with glibenclamide (GB), showed reduced blood-glucose levels as compared to control group.

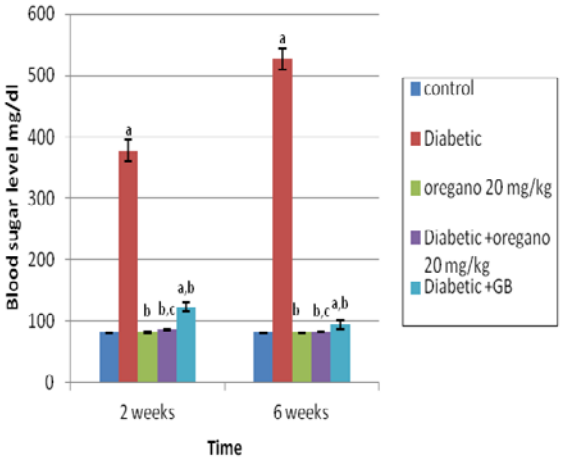

Figure 1. Effect of aqueous extracts of oregano leaves (20 mg/kg) on blood-sugar level (mg/dl) - Data are expressed as the mean ± SE. Each value corresponds to a mean of 6 animals. a, comparison of Group I vs Group II .b ,comparison of Group III, Group IV & Group V vs Group II. c, comparison of Group IV vs Group V at *P<0.05*.

In the untreated diabetic animals, the initial HbA1C value increased significantly (3.634 ±0.0093) compared to the control. In the oregano treated diabetic groups (20 mg/kg) the HbA1C value return to the normal value as compared to control group (Fig.2).

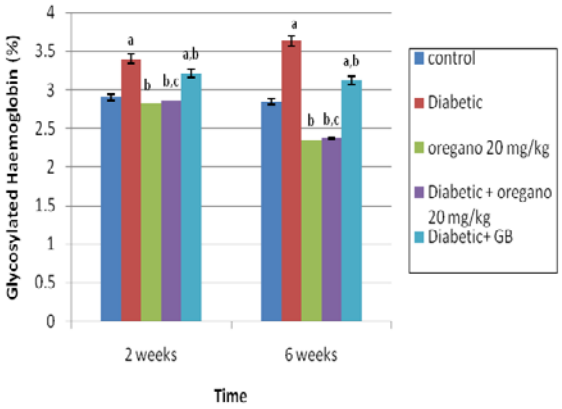

Figure 2. Effect of aqueous extracts of oregano leaves (20 mg/kg) on glycosylated haemoglobin (%) - Data are expressed as the mean ± SE. Each value corresponds to a mean of 6 animals. a, comparison of Group I vs Group II .b ,comparison of Group III, Group IV & Group V vs Group II. c , comparison of Group IV vs Group V at *P<0.05*.

On the other hand, the serum insulin levels decreased markedly after STZ administration. Oral administration of the aqueous OV extracts (20 mg/kg) or GB partially improved insulin levels in STZ diabetic rats ($P < 0.05$) after 2 weeks. After 6 weeks, oral administration of the aqueous OV extracts (20 mg/kg) significantly improved insulin levels in STZ diabetic rats ($P < 0.05$) in comparison with standard drug GB as shown in figure 3.

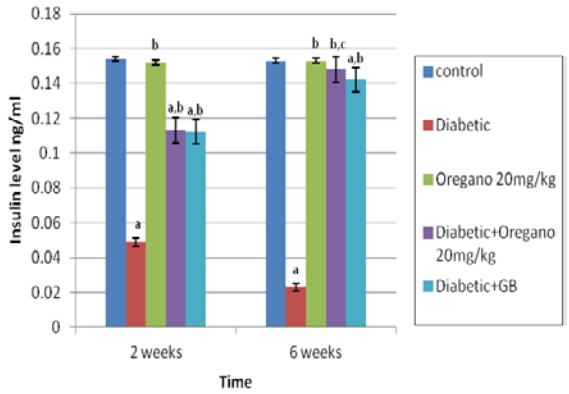

Figure 3. Effect of aqueous extracts of oregano leaves (20 mg/kg) on Insulin (ng/ml). Data are expressed as the mean ± SE. Each value corresponds to a mean of 6 animals. a , comparison of Group I vs Group II .b ,comparison of Group III, Group IV & Group V vs Group II. c , comparison of Group IV vs Group V at *P<0.05*.

Figures 4 and 5 revealed marked depletion in the liver and muscle glycogen contents ($P < 0.05$) in STZ-induced diabetic rats compared to control. The administration of OV (20 mg/kg) or GB for six weeks significantly ($P < 0.05$) increased the liver and muscle glycogen contents in diabetic rats compared to untreated diabetic and control groups. It was also noticed that OV (20 mg/kg) control groups showed a significant increase in liver and muscle glycogen contents (3.12 ± 0.174 and 0.594 ± 0.0209) throughout the experimental period.

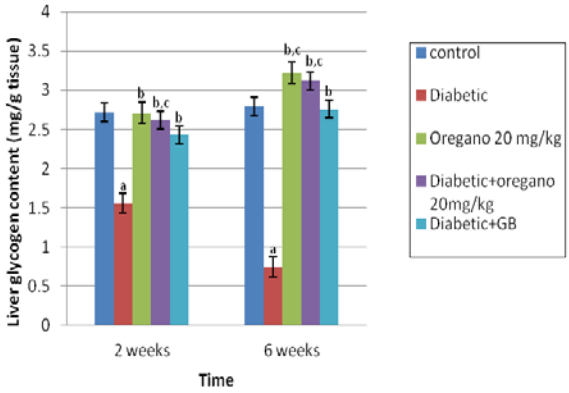

Figure 4. Effect of aqueous extracts of oregano leaves (20 mg/kg) on Liver glycogen content (mg/g tissue) - Data are expressed as the mean ± SE. Each value corresponds to a mean of 6 animals. a, comparison of Group I vs Group II .b, comparison of Group III, Group IV & Group V vs Group II. c, comparison of Group IV vs Group V at *P<0.05*.

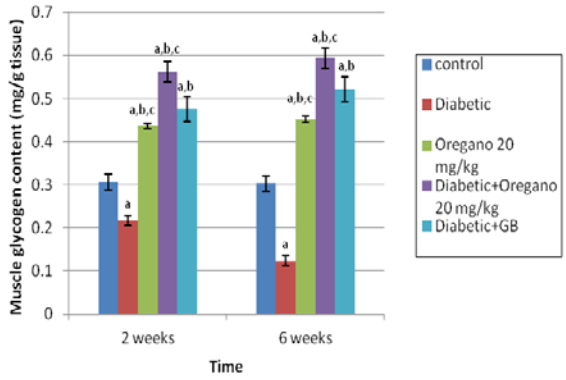

Figure 5. Effect of aqueous extracts of oregano leaves (20 mg/kg) on muscle glycogen content (mg/g tissue) - Data are expressed as the mean ± SE. Each value corresponds to a mean of 6 animals. a, comparison of Group I vs Group II .b, comparison of Group III, Group IV & Group V vs Group II. c, comparison of Group IV vs Group V at *P<0.05*.

In the STZ-treated groups, the pancreatic amylase level was significantly increased. Oral administration of the aqueous OV extracts (20 mg/kg) administration produced a significant decrease in pancreatic amylase levels in STZ diabetic rats ($P < 0.05$) as compared to control group (Table 1).

Table1. Effect of aqueous extracts of oregano leaves (20 mg/kg) on Pancreatic amylase.

parameter / Group	Pancreatic amylase(U/L)	
	2nd week	6th week
Control	243.80 ±0.707	242.80 ± 0.735
Diabetic	365.42 ± 2.315 [a]	287.20 ± 0.583 [a]
Oregano(20 mg/kg)	235.20 ±0.860 [a, b]	237.60 ± 0.927 [b]
Diabetic + Oregano(20 mg/kg)	232.20 ± 0.718 [a, b]	246.00 ± 1.140 [b,c]
Diabetic + GB	242.20 ± 1.772 [b]	252.60 ±0.926 [a, b]

- Data are expressed as the mean ± SE. Each value corresponds to a mean of 6 animals. a, comparison of Group I vs Group II .b, comparison of Group III, Group IV & Group V vs Group II. c, comparison of Group IV vs Group V at *P<0.05*.

The mean body weight of rats was shown in table 2. Results showed that weight of diabetic controls significantly decreased during the experimental period while normal controls and OV extract rats gained significant weight ($P < 0.05$). The diabetic group given oregano leaves extract (20 mg/kg) or GB administration maintained weight gain. The liver weight/body weight ratios have been shown to increase in diabetic rats significantly ($P < 0.05$) in comparison to control animals (Table 2). This increase was completely reversed by OV leaves extract (20 mg/kg) administration and partially improved by GB. STZ-induced diabetes caused a significant ($P < 0.05$) increase in kidney weight/body weight ratios in comparison to control. This enhancement was partially improved by OV (20 mg/kg) or GB (Table 2).

Table 2. Effect of aqueous extracts of oregano leaves on body weight ,liver and kidney weights/body weight ratios in rats

parameter / Group	body weight (g)		Liver weight /ody weight		Kidney weight / body weight	
	2nd week	6th week	2nd week	6th week	2nd week	6th week
Control	210.00 ± 1.303	235.00 ± 1.612	0.03268 ±0.00029	0.03278 ±0.00025	0.00420 ±0.00049	0.00419 ±0.00005
iabetic	186.00 ± 4.703 a	172.00 ± 1.327 a	0.03992 ±0.00062 a	0.05273 ±0.00115 a	0.00602 ±0.00022 a	0.00612 ±0.00026 a
regano (20 mg/kg)	205.00 ± 1.581 b	236.00 ± 1.095 b	0.03042 ±0.00013 b	0.03321 ±0.00070 b	0.00414 ±0.00004 b	0.00412 ±0.00010 b
iabetic +Oregano (20 mg/kg)	202.00 ± 1.140 b,c	225.00 ± 2.509 b,c	0.03290 ±0.00024 b,c	0.03268 ±0.00738 b,c	0.00519 ±0.00010 a, b	0.00490 ±0.00043 a, b
iabetic + GB	196.00 ± 1.673 a	211.00 ±2.881 b	0.03814 ±0.00153 a, b	0.04267 ±0.02059 a, b	0.00544 ±0.00021 a, b	0.00493 ±0.00010 a, b

- Data are expressed as the mean ± SE. Each value corresponds to a mean of 6 animals. a, comparison of Group I vs Group II .b, comparison of Group III, Group IV & Group V vs Group II. c, comparison of Group IV vs Group V at $P<0.05$.

The results in Table 3 showed significant increase ($P < 0.05$) in the level of urea, uric acid and creatinine, which are markers of renal dysfunction in the diabetic group compared to control group. This enhancement was partially improved by OV (20 mg/kg) or GB (Table 3).

Table 3. Effect of aqueous extracts of oregano leaves on urea , uric acid and creatinine in rats

parameter / Group	Urea (mg/dl)		Uric acid (mg/dl)		Creatinine (mg/dl)	
	2nd week	6th week	2nd week	6th week	2nd week	6th week
Control	30.40 ±2.461	30.60 ±2.021	1.24 ±0.040	1.30 ±0.032	0.30 ±0.010	0.31 ±0.020
Diabetic	59.21 ±6.406 a	65.43 ±5.137 a	1.94 ±0.323 a	2.52 ±0.902 a	0.72 ±0.096 a	0.94 ±0.137 a
Oregano (20 mg/kg)	32.00 ±3.284	33.00 ± 3.153	1.32 ±0.583	1.34 ±0.058	0.32 ±0.157	0.33 ±0.155
Diabetic +Oregano (20 mg/kg)	53.00 ±2.030 a, b	49.00 ±2.144 a, b	1.84 ±0.340 a, b	1.60 ±0.045 a, b	0.65 ±0.030 a, b	0.56 ±0.144 a, b
Diabetic + GB	55.00 ±3.112 a, b	52.20 ±3.138 a, b	1.86 ±0.082 a, b	1.65 ±0.178 a, b	0.67 ±0.112 a,b	0.58 ±0.138 a, b

- Data are expressed as the mean ± SE. Each value corresponds to a mean of 6 animals. a, comparison of Group I vs Group II .b, comparison of Group III , Group IV & Group V vs Group II. c, comparison of Group IV vs Group V at $P<0.05$.

4. Discussion

Inspite of the presence of known antidiabetic medicine in the pharmaceutical market, herbal drugs are frequently considered to be less toxic and also free from side effects, than synthetic ones. In the present study, the administration of streptozotocin (45mg/kg) induced hyperglycaemia in rats. Treatment of diabetic rats with oregano at 20 mg/kg showed a significant decrease in the blood-sugar level. This may be due to that oregano enhance the insulin sensitivity of the receptors on cells, leading to reduced levels of blood sugar and more energy production. The accumulating evidence suggests that modulation of insulin secretion and/or insulin action mechanisms could be involved in the antidiabetic effect of oregano. This evidence was confirmed with Talpur *et al.* (2005) who reported that extracts of oregano improve blood sugar levels by enhancing insulin sensitivity. Also,the hypoglycaemic effect of oregano may be due to the interference on absorption of dietary carbohydrates in small intestine or stimulation of glucose utilization by peripheral tissues. In line with this evidence of this study, Maghrani *et al.* (2003) and Ortiz-Andrade *et al.* (2007) reported that the hypoglcaemic action of medicinal plant may be due to a reduction in the intestinal absorption of glucose and/or inhibiton of tubular glucose reabsorption. Oregano leaves contain phenolic glucosides that help control blood sugar as reported by Takeda *et al.* (2008).

The level of glycosylated haemoglobin (HbA1C) has been shown to be an important parameter of chronic glycaemic control in patients with DM, an elevated HbAlc almost always indicates DM (The International Expert Committee, 2009). The present data showed that, the high levels of HbAlc in diabetic rats were significantly lowered by the treatment with aqueous extract of oregano leaves. Decreased HbA1C levels in the treated diabetic rats could be due to an improvement in insulin secretion from the remnant pancreatic β-cells in diabetic rats, consequently, resulting in improvement in glycemic control (Vinay *et al*., 2010).

Increased insulin level in diabetic rats after treatment with the oregano treatment (Fig. 3) this may be due to the bioactive molecules present in oregano leaf extract that may probably stimulate the β cells of the pancreas to produce insulin. Furthermore, this effect may be due to that oregano has been shown to have an insulin-like biological activity. This explanation agrees with that reported by Broadhurst *et al.* (2000) who showed that the positive effects of oregano extracts on insulin activity suggest a possible role of this plant in improving glucose and insulin metabolism. In contrast, Lemhadri *et al*. (2004) concluded that aqueous extract of OV (20 mg/kg) exhibits an anti-hyperglycaemic activity in STZ rats without affecting basal plasma insulin concentrations.

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues, especially skeletal muscles are direct reflection of insulin activity. The observed decrease in hepatic and muscle glycogen may be due to insufficient insulin and inactivation of the glycogen synthetase system in the diabetic state (Vinay *et al*., 2010). However, after the treatment with oregano, there was a significant increase in the liver and muscle glycogen levels

in the diabetic rats. The increased hepatic glycogen level in the treated diabetic rats may be due to increased level of insulin, which has increased glycogenesis and decreased glycogenolysis and gluconeogenesis. Thus antihyperglycemic effect of OV may be due to protection of surviving pancreatic β cells, increase in insulin secretion and glycogen storage (Jagtap & Patil, 2009). Inhibitory effects on glycogenolysis have been reported for glibenclamide in the presence of insulin after stimulation of glycogenolysis by glucagon (Carvalho-Martini *et al*., 2006).

The increment in pancreatic amylase in diabetic rats was antagonized by oregano treatment. The inhibition of pancreatic amylase delay carbohydrate digestion and protract overall carbohydrate digestion time, resulting in the reduction in glucose absorption rate and consequently, dulling the postprandial plasma glucose rise. Several indigenous medicinal plants have a high potential in inhibiting pancreatic amylase enzyme activity (Valiathan, 1998). Mc Cue *et al*. (2004) reported that extracts of clonal oregano lines have strong inhibitory activity against porcine pancreatic amylase (PPA) *in vitro*. MC Cue and Shetty (2004) reported the ability of rosmarinic acid, one of the principal phenolic components of oregano, to inhibit porcine pancreatic amylase (PPA) activity. One of the potentially important components of anti-diabetic activity by oregano extracts is mild amylase inhibition by phenolic antioxidants that contribute management of hyperglycemia (Mc Cue *et al*., 2004).

Weight loss has been known to be one of the symptoms of DM. Similar observations were detected in many experimental studies (Al-Attar & Zari, 2007; Subash-Babu *et al*., 2008; Sellamuthu *et al*., 2009 and Salahuddin *et al*., 2010). In the diabetic control rats, deficiency of insulin led to decreased amino acids uptake by tissues with a consequent reduction in the level of protein synthesis. Also insulin deficiency results in lipolysis in adipose tissues and protein breakdown (Vasudenvan & Sreekumari, 2007). The increase in weight observed in the group treated with oregano, and normal control group may be a reflection of efficient insulin action. The liver and kidney weights /body weight ratios in the diabetic groups were higher than those of the normal groups (Table, 2) suggesting the occurrence of the edema and inflammation of these organs as reported by Kamath and Rajini (2006). High concentrations in serum urea, uric acid, and creatinine strongly suggested impairment of kidney function in diabetic rats. Similar effect was recorded previously by Alarcon *et al*. (2005) Jaya *et al*. (2010) and Manikandaselvi *et al*. (2012). The present data indicated that the oregano supplement has a partial renoprotective effect. These results are in agreement with other previous studies by Khan *et al*. (2011) who stated that oregano showed antiurolithic activity both *in vitro* and *in vivo* models in addition to its antioxidant, renal epithelial cell protective, antispasmodic and diuretic activities. These different activities observed in the crude extract might be due to the presence of flavonoids that were known to possess diuretic activities (Ramamoorthy *et al*., 2010).

Oregano has long been used in traditional medicine in the treatment of common ailments and have been potential for positive modulation of oxidation-linked diseases such as diabetes. Flavonoids are considered as active principles in many medicinal plants and natural products with a positive effect for human health (Wollenweber, 1988). These natural compounds could act separately or synergistically to cause the hypoglycaemic effect. This could not exclude the intervention of other phytochemical constituents as bioactive hypoglycaemic agents. Most of oregano's effects on the body are due to its high content of antioxidants, which play a role in destroying the production of free radicals (Spiridon *et al*., 2011) and modulates blood sugar and lipids (Singletary, 2010).

5. Conclusion

The obtained results may support the use of oregano as culturally adopted treatments for insulin resistance and hyperglycemia and support its inclusion as a natural, safe, anti-diabetic therapy for modulation of Type 2 diabetes mellitus.

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