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Modulatory effect of *Origanum majorana* leaves extract against diabetic induced changes in spleen of adult rats

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ABSTRACT

For decades, traditional plant medicines have been utilized to treat diabetes. In traditional medicine, *Origanum majorana* L., known as sweet marjoram, is used to cure a variety of illnesses. It belongs to the Lamiaceae family. The purpose of the current study was to evaluate the ameliorative and antihyperglycemic effects of *O. majorana* leaves extract (OMLE) on the splenic tissue of diabetic rats. The following four groups (n=10) of adult male albino rats were divided as; group (Gp1) was used as the negative control. For four weeks, Gp2 had been administered OMLE (20 mg/kg b.wt) orally every day. Streptozotocin (STZ) (40 mg/kg b.wt) was injected intraperitoneally (i.p.), once, into Gp3 and Gp4. OMLE (20 mg/kg b.wt) was subsequently given orally to Gp4 only daily for four weeks. Blood glucose and insulin levels, superoxide dismutase (SOD) and catalase (CAT) activities, reduced glutathione (GSH) and malondialdehyde (MDA) levels, and white blood cell count (WBCs) were measured. Spleen tissues were investigated histologically. Diabetic rats had a noteworthy increase in blood glucose and MDA levels, accompanied by a noteworthy fall in plasma insulin levels, SOD, and CAT activities, white blood cell count and GSH level ($p < 0.01$). The findings showed that the spleen tissue of diabetic rats had many histological alterations. The biochemical indicators and histological structure significantly improved when diabetic rats were treated with OMLE. In conclusion, OMLE may have a protective effect and reduce diabetic complications in the spleen tissues of diabetic rats.

Keywords:
Antioxidant, Glucose, Histopathological, Insulin, *Origanum majorana*, Rats, Spleen, Streptozotocin.

1. Introduction

A range of metabolic disorders with several etiologies include diabetes mellitus (DM) according to Catalano et al. (2023), it is characterized by chronic hyperglycemia and disruption of the metabolism of proteins, fats, and carbohydrates because of a deficiency in insulin secretion or activity. Diabetes has a wide range of effects on the body and is linked to major side effects including renal failure, heart disease, stroke, blindness, and amputation of the lower limbs (IDF, 2016; Mobasher et al., 2021). According to estimates, there are currently 40 million diabetic people; by 2025, that figure could rise to 74 million (Dogan et al., 2015). Medicinal plants showed potential antidiabetic activity (El-Said et al., 2019). Sweet majorana, *Origanum majorana* L., is an aromatic plant that is highly valuable in the industrial, medicinal, and economic domains. It is a member of the Lamiaceae family (Bina et al., 2017). This plant has been shown to have a wide range of pharmacological including antitumor, antibacterial, and antifungal, antioxidant,

hepatoprotective, cardioprotective, gastroprotective, and anticholinesterase inhibitory properties (Lemhadri et al., 2004; Ocana-Fuentes et al., 2010; Sharma et al., 2011). Bioactive components like terpinen-4-ol, cis-sabinene hydrate, p-cymene, sabinene, trans-sabinene hydrate, and terpineol have been found to be present in *O. majorana* essential oil. Carvacrol and thymol were *O. majorana's* two most notable chemical constituents (Freire et al., 2011). It contains phenolic terpenoids, flavonoids, tannins, and phenolic glycosides (Al-Howiring et al., 2009). *O. majorana* leaves and floral parts have been shown to contain a variety of chemicals, including phenolic derivatives, vitamin A, K, C, magnesium, and manganese, which can be extracted in both aqueous and methanol form (Baatour et al., 2011). This study set out to determine the antihyperglycemic action of *O. majorana* leaves extract (OMLE) as well as its ameliorative effect on the diabetic rats' splenic tissues.

2. Materials and Methods

Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich Chemical, located in Steinheim, Germany. The rat insulin ELISA kit was bought from Millipore USA (EZRMI-13K. Glucose, reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA), kits purchased from Biodiagnostic, Egypt.

Preparation of *O. majorana* leaves aqueous extract

The Benavides et al. (2010) procedure was followed in the preparation of the *O. majorana* leaves extract (OMLE). *O. majorana* leaves were purchased from the Saudi Arabian market after being certified and recognized by a professional botanist. To produce OMLE, three hundred grams of *O. majorana* leaves were chopped in water, boiled in 1.5 liters of distilled water for 15 minutes at 60 ^{o}C, and then filtered and kept at -20 ^{o}C (Mohamed and Nassier, 2013).

Experimental animals

Forty mature male wistar albino rats, weighing 120 ± 7 g, were acquired from the Alexandria University's Faculty of Medicine in Alexandria, Egypt. All rats were kept under the same environmental conditions for one week before the study. They were fed on a normal rat pellet diet and given access to water in a temperature-controlled environment with a 12-hour light/dark cycle. The National Institutes of Health's (NIH) Public Health Guide for the care and use of laboratory animals gave its approval for all animal experiments.

Induction of type 2 diabetes

Animals were assigned to a high fat diet (HFD). After 10 days of the HFD, overnight-fasted (12 h) rats were injected with a single injection of freshly prepared STZ (40 mg/kg, intraperitoneally (i.p), in 0·1 M-citrate–phosphate buffer, pH 4·5). The development of hyperglycemia in rats was confirmed by estimation of fasting serum glucose 72 h after STZ injection. Rats with fasting serum glucose level above 13·89 mmol/l were considered diabetic according to Sharma et al. (2011).

Experimental design

Four groups of rats (n= 10) were divided as follows: Gp1 had only taken distilled water served as negative control. Gp2 received daily OMLE (20 mg/kg b.wt) orally for four weeks. Following an i.p injection of STZ (40 mg/kg b.wt) by Gp3 and Gp4, Gp4 only had given OMLE (20 mg/kg b.wt) orally daily for four weeks.

Blood collection and biochemical analysis

Under little anesthesia (isoflurane), the rats were slaughtered. Blood samples were collected from all groups by cardiac puncture into either with or without ethylene-diamine terta acetic acid anticoagulant. Blood was drawn, left two hours at room temperature to clot, centrifugation was performed for ten minutes. Samples of sera were kept at -20 ^{o}C for biochemical analysis. Tietz (1995) method was followed in determining serum glucose levels. The quantification of insulin in rat serum was done non-radioactively using a Millipore USA rat insulin ELISA kit (Weyer et al., 2000). SOD activity was measured according to Nishikimi et al. (1972). MDA level was evaluated using

Ohkawa et al. (1979) method. GSH level was determined using Beutler et al. (1963) method. CAT activity was assessed according to Aebi (1984). Coles (1980) method was followed in determining the total white blood cell (WBC) count.

Preparation of spleen for histological investigation

For the histology studies, portions of the spleen tissues were separated right away, cleaned in cold saline solution, and then preserved in 10% neutral buffered formalin (Lulat et al., 2016). 4-5 µm sections of the collected sections were stained with hematoxylin and eosin for histopathological examination under a light microscope (Optica light microscope, B-350) (Bancroft and Gamble, 2008).

Statistical analysis

The reported values are given as mean ± SD. A one-way analysis of variance test (ANOVA) was used to examine the statistical significance of the difference between the groups. According to Kotz et al. (2006), results were deemed substantially different when the p value was less than 0.01.

3. Results

Treatment of diabetic rats with OMLE ameliorated glucose, insulin levels and WBCs count

Rats in the diabetic group (a group administered STZ) had significantly higher blood glucose levels than the control group ($p < 0.001$) (Table 1). When compared to the diabetic group, the diabetic rats treated with OMLE had significant reduction in blood glucose levels ($p < 0.001$). When the diabetic rats were treated with OMLE, their insulin levels dramatically improved ($p < 0.001$), although they still showed a significant drop in the diabetic group compared to the control group. Table 1 indicates that the diabetic group's total WBC count significantly decreased, whereas the diabetic rats treated with OMLE showed a substantial increase in total WBC count compared to the control group ($p < 0.001$).

Effect of treatment with OMLE on the antioxidants/oxidant's biomarkers in diabetic rats

The results showed that SOD, CAT activities, and GSH level in diabetic rats were significantly lower than in control rats. When diabetic rats were treated with OMLE, there was a significant increase in the activity of antioxidant enzymes ($p < 0.001$) (Figs. 1 and 2). On the other hand, compared to the control group, the diabetic group's MDA level was shown to be significantly higher. The treatment of the diabetic group with OMLE similarly considerably decreased the MDA levels ($p < 0.001$) (Fig. 1B).

Table 1. Effect of OMLE on the glucose, insulin level and the total WBCs count in different groups

Groups	Glucose (mg/dl)	Insulin (µIU/ml)	WBCs count (× 10^3 mm^3)
Ctrl.	110 ±7.9	13.5 ± 0.49	5.29 ± 0.45
Ctrl. /OMLE	116 ± 4.7	13.3 ± 0.32	5.38 ± 0.54
Diabetic	267^{a***} ± 5.9	5.08^{a***} ± 0.55	1.94^{a}***± 0.51
Diabetic/OMLE	116^{b***} ± 6.8	12.47^{b**} ± 0.38	4.66^{b***} ± 0.58
Change %	-56.39	145.47	140.21

The values represented means ± S.D; **Ctrl:** Control; **OMLE:** *O. majorana* leaves extract. **a**: Statistically significant with Ctrl.; **b**: Statistically significant with diabetic; ***: Statistically significant at $p \leq 0.001$.

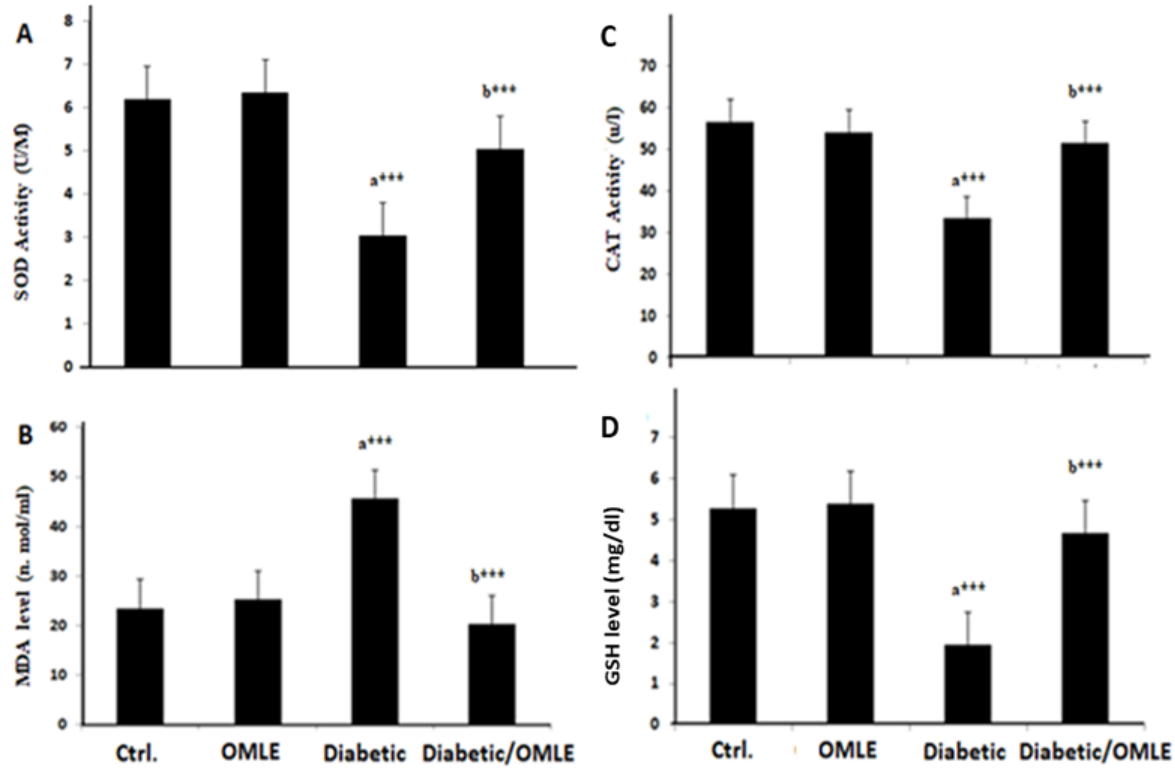

Fig. 2. Effect of *O. majorana* leaves extract on SOD activity (A) and MDA level (B), CAT activity(C) and GSH level (D). OMLE: *Origanum majorana* leaves extract; a: Statistically significant with Ctrl.; b: Statistically significant with diabetic; ***: Statistically significant at $p \leq 0.001$.

Histological observation of spleen sections

The control rats (Gp1) had normal red and white pulp in their spleen tissues, according to histological analysis. The germinal core was clearly distinguished, and the marginal zone was evident (Fig. 3 A and B). The spleen tissues of rats given OMLE (Gp2) analysis revealed typical red and white pulp, a clear marginal zone, and a well-differentiated germinal center (Fig. 3 C and D). Vacuolated red and white pulp was visible in the spleen tissues of diabetic rats (Gp3), and clustered nuclei were seen in the marginal zone. The germinal center showed deteriorated cytoplasm, and the central artery also showed degeneration (Fig. 4 A). Rats with diabetes had vacuolization in the marginal zone of their spleen, and their red pulp had clustered nuclei. In the venous sinus, degenerated nuclei are seen, and white pulp also shows vacuolated gaps (Fig. 4 B). The spleen tissues of diabetic rats administered OMLE (Gp4) exhibited more restoration in the cytoplasmic material of the red pulp with few vacuolated areas, marginal zone. The spleen tissues of diabetic rats administered OMLE (Gp4) exhibited more restoration in the cytoplasmic material of the red pulp with few vacuolated areas, the marginal zone, the nuclei, and the structure of the white pulp. There were noticeable trabecular veins (Fig. 4 C and D).

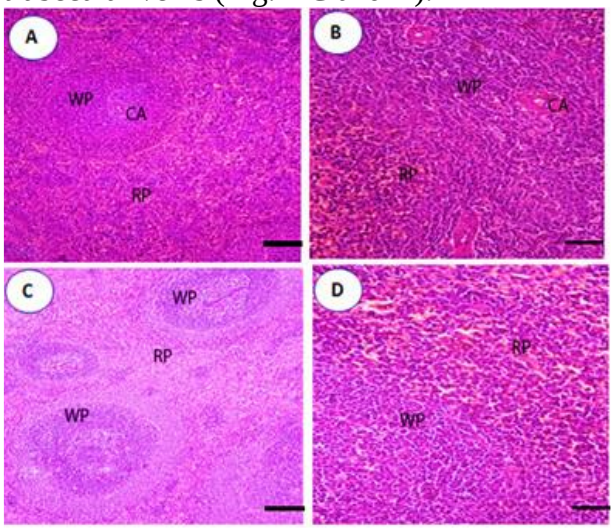

Fig.2. Photomicrograph of spleen section of different rats' groups. Normal group showing normal red pulp (RP), normal white pulp (WP) and normal central artery (CA) (A and B), normal rats

treated with OMLE showing normal red pulp and white pulp (C and D). (H&E stain) (X 200,400).

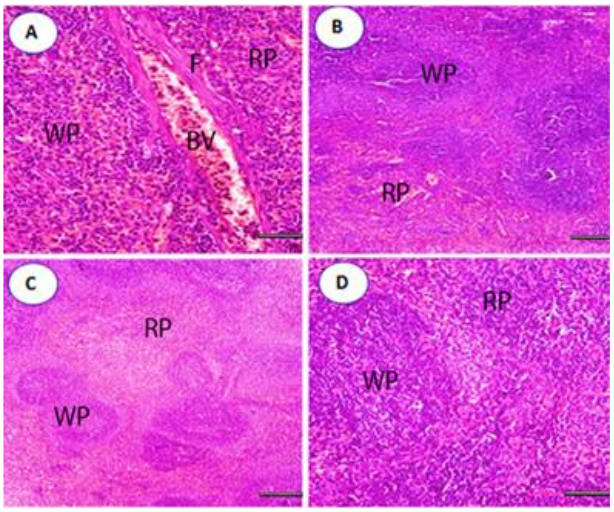

Fig. 3. Photomicrographs of spleen sections of diabetic rats' groups. Diabetic group showing vacuolated red pulp (RP) and white pulp (WP), vacuolization in marginal zone and white pulp, accumulation of the dense branched fibers (F) and Dilatation and congestion of the blood vessels (BV) (A and B). While diabetic rats treated with OMLE0 show more restoration in structure of white pulp, marginal zone, nuclei, and cytoplasmic material of red pulp with few vacuolated spaces. Trabecular veins are distinct (C and D). (H&E stain) (X 200,400).

4. Discussion

Medicinal plants have been used for centuries in the treatment of diabetes as potential sources of hypoglycemic agents (Pandey et al., 2011). The present study reported that the insulin level was decreased, and blood glucose level was increased in diabetic rats. These changes could be due to the destruction of pancreatic β cells by STZ. STZ induces diabetes probably through the generation of free radicals (Gupta et al., 2004). Treatment of diabetic rats with OMLE decreased the glucose level and increased insulin levels. These findings agreed with Kamel. (2014), who reported that OMLE had hypoglycemic activity. The mechanism of action of OMLE could be due to stimulation of insulin secretion from pancreatic β-cells (Proks et al., 2002). A significant decrease in the total WBCs count was reported in the diabetic rats, however, a significant increase in the total WBCs count was reported in diabetic rats which had treated with OMLE. These findings are in consistent with Lemhadri et al. (2004), who reported that the OMLE augmented WBCs changes. Diabetes Mellitus increases oxidative stress and reduces antioxidant status (Caturano et al., 2023). An increase in the MDA level was reported in diabetic rats which could be due to the increase of oxidative stress (Pitocco et al., 2010). The treatment of diabetic rats with OMLE showed a decline in MDA level. This finding agreed with Abdel-Massih et al. (2010) who reported that OMLE inhibited lipid peroxidation by exhibiting a strong scavenging activity. A significant decrease in the GSH level, SOD and CAT activities were noticed in diabetic rats, however, treatment of diabetic rats with OMLE returned these values were close to normal. The SOD, CAT activities and GSH level were shown to be low in diabetic rats, which is in line with the findings of Guo et al. (2014). Furthermore, it was reported by Baatour et al. (2011) that phenolic and flavonoid chemicals found in OMLE may be the cause of its antioxidant action. According to the histopathology results, there were no appreciable alterations in the splenic tissues' histological structures between the normal rats given OMLE and the control group. Rats with diabetes displayed several histological structural alterations in their splenic tissues. These results are in line with those of Ebaid et al. (2015), who discovered that diabetes was associated with several histological alterations in the splenic tissues. These results also support the findings of Park et al. (2013), who found that oxidative stress and diabetic complications induce cell apoptosis in vital organs. Treatment of diabetic rats, however, with OMLE ameliorated these effects that induced in splenic tissues of diabetic rats. The protective effect of OMLE could be due to the presence of antioxidant compounds (Botsoglou et al., 2002). These results are in accordance with a previous study by Al Syaad and Ibrahim (2020), who found that OMLE may be useful in protecting vital organs. In conclusion, treatment with OMLE decreased the hyperglycemic conditions and ameliorated the adverse effect of diabetes on the splenic tissues.

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Conflict of interest

All authors declared that there was no conflict of interest.

Author contributions

The authors have equal contribution. All authors read and approved the final manuscript.

5. References

Aebi H ,1984. Catalase *in vitro*. Meth. Enzymol. 105: 121–126.

Al-Howiring T, Alsheikh A, Algasoumi S, Al-Yahyia M, Eltahir K, Rafatullah S, 2009, Protective effects of Organiummajorana L. at various models of gastric mucosal injury in rats. Am. J. Chin. Med. 37: 351-355.

Al Syaad KM, Ibrahim EH, 2020. Study of the antioxidant, immunomodulatory and antibacterial properties of Origanum majorana leaf acetone extract. Pak. J. Pharm. Sci. 33: 2209-2218.

Baatour O, Kaddour R, Mahmoudi H, TarchounI, Bettaieb I, Nasri N, Mrah S, Hamdaoui G, Lachaal M and Marzouk B, 2011.Salt effects on *Origanum majorana* fatty acid and essential oil composition. J. Sci. Food Agric. 10: 1002-4495.

Benavides V, D'Arrigo G and Pino J, 2010. Effects of aqueous extract of Origanum vulgare L. (Lamiaceae) on the preimplantational mouse embryos. Rev. Peru. Biol. 17: 381-384.

Beutler E, Duron O, Kelly, MB J, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61: 882-8.

Bina F, Rahimi R, 2017. Sweet marjoram: a review of ethnopharmacology, phytochemistry, and biological activities. J. Evid. Based Complementary Altern. Med. 22:175-185.

Botsoglou NA, Florou-Paneri P, Christaki E, Fletouris DJ, Spais AB, 2002. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. Br. Poult. Sci. 43: 223-230.

Caturano A, D'Angelo M, Mormone A, Russo V, Mollica MP, Salvatore T, Galiero R, Rinaldi L, Vetrano E, Marfella R, Monda M, Giordano A, Sasso FC, 2023. Oxidative Stress in Type 2 Diabetes: Impacts from Pathogenesis to Lifestyle Modifications. Curr. Issues Mol. Biol. 45: 6651-6666.

Coles H, 1980. Veterinary Clinical Pathology 3rd ed., W.B. Saunder Co. Philadelphia.

Dogan A, Celik I, Kaya MS, 2015. Antidiabetic properties of lyophilized extract of acorn (*Quercus brantii* Lindl.) on experimentally STZ-induced diabetic rats. J. Ethno-pharmacol.176: 243-251.

Ebaid H, Al-Tamimi J, Metwalli A, Allam A, Zoheir K, Ajarem J, Rady A, Alhazza I, Ibrahim K, 2015. Effect of STZ-Induced Diabetes on Spleen of Rats: Improvement by Camel Whey Proteins. Pak. J. Zool. 47: 1109-1116.

El-Said KS, Tarek M, Amira E. 2019. Anti-diabetic activity of egyptian celery apigenin. Asian Journal of Dairy and Food Research. 38.

Freire JM, Cardoso M G, Batista L R and Andrade M A, 2011. Essential oil of *Origanum majorana* L., *Illicium verum* Hook.f. and *Cinnamomum zeylanicum* Blume, chemical and antimicrobial characterization. Rev. Bras. Pl. Med. Botucatu.13: 209-214.

Guo C, Zhang C, Li L, Wang Z, Xiao W, Yang Z, 2014. Hypoglycemic and hypolipidemic effects of oxymatrine in high-fat diet and streptozotocin-induced diabetic rats. Phytomedicine. 21:807–814.

Gupta S, Kataria M, Gupta PK, Murganadan S, Yashroy RC, 2004. Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. J. Ethnopharmacol. 90: 185-189.

IDF, (2014): International Diabetes Federation. Diabetes Atlas.3thed© implication. Am J Transl Res. 2: 316-31.

Kamel MA, 2014. Protective effects of marjoram oil (Organium majorana L.) on antioxidant enzymes in experimental diabetic ratsAssiut Vet. Med. J. Vol. 60: 68-75.

Kotz S, Balakrishnan N, Read CB, Vidakovic B, 2006. Encyclopedia of statistical sciences. 2nd ed. Hoboken, Wiley-Interscience, N.J.

Lemhadri A, Zeggwagh NA, Maghrani M, Jouad H and Eddouk M, 2004. Anti hyperglycaemic activity of aqueous extract of Origanum vulgare growing wild in Tafilate region. J. Ethnopharmacol. 92:251-256.

Lulat SI, Yadav YC, Balaraman R, Maheshwari R, 2016. Antiurolithiatic effect of lithocare against ethylene glycol-induced urolithiasis in Wistar rats. Indian J. Pharmacol. 48:78–82.

Mobasher MA, Germoush MO, Galal El-Tantawi H, Samy El-Said K. 2021. Metformin improves biochemical and pathophysiological changes in hepatocellular carcinoma with pre-existed diabetes mellitus rats. Pathogens. 10(1): 59.

Mohamed NA and Nassier OA, 2013. The Antihyperglycaemic Effect of the aqueous extract of Origanum vulgare leaves in Streptozotocin-Induced Diabetic rats. Jordan J. Biol. Sci. 6:31-38.

Nishikimi M, Roa NA, Yogi K, 1972. Biochem. Bioph. Res.Common., 46; 849 – 854.

Ocana-Fuentes A, Arranz-Gutierrez E, Senorans F, Reglero G, 2010. Supercritical fluid extraction of oregano (Origanum vulgare) essentials oils: anti-inflammatory properties based on cytokine response on THP-1 macrophages. Food Chem. Toxicol. 48:1568- 1575.

Ohkawa H, Ohishi W, Yagi K, Anal, 1979. Biochem .95: 351.

Pandey A, Tripathi P, Pandey R, Srivatava R, Goswami S, 2011. Alternative therapies useful in the management of diabetes: A systematic review. J. Pharm. Bioallied. Sci.3:504-12.

Park, GB, Kim YS, Lee HK, Cho DH, Kim D, HUR DY, 2013. CD80 (B7.1) and CD86 (B7.2) induce EBV-transformed B cell apoptosis through the Fas/FasL pathway. Int. J. Oncol. 43: 1531-1540.

Pitocco D, Zaccardi F, Di Stasio E, Romitelli F, Santini SA, Zuppi C, 2010. Oxidative stress, nitric oxide, and diabetes. Rev. Diabet Stud.7:15-25.

Proks P, Reimann F, Green N, Gribble F, Ashcroft F, 2002. Sulfonylura stimulation of insulin secretion. Diabetes. 51: S368-S376.

Sharma Ak, Bharti S, Ojha S, Bhatia J, Kumar N, 2011. Up-regulation of PPARgamma, heat shock protein-27 and -72 by naringin attenuates insulin resistance, beta-cell dysfunction, hepatic steatosis and kidney damage in rat model of type 2diabetes. Br. J. Nutr. 106: 1713-1723.

Tietz NW, 1995. Clinical guide to laboratory tests. 3rd Ed.Philadelphia: WB Saunders: 268-273.

Weyer C, Hanson RL, Tataranni PA, Bogardus C, Pratley RE, 2000. high fasting plasma insulin concentration Predicts type 2 diabetes independent of insulin resistance. Diabetes. 76: 2094-101.