

Effect of *origanum vulgare* extract on glucose level and some parameters of immunity in alloxane diabetic mice

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تأثير مستخلص نبات المردقوش على مستوى الكلوكوز وبعض معايير المناعة في الفئران المستحدث فيها السكري باستخدام الالوكسان

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الخلاصة

ان الهدف من البحث ، دراسة تأثير مستخلص نبات المردقوش على مستوى السكر في الدم ، الصورة الدموية (العدد الكلي والتفريقي لكريات الدم البيض) وعلى الاضداد (IgG , IGA , IGM) والمتمم C₃,C₄) في الفئران المستحدث فيها داء السكري بالالوكسان . قسمت الحيوانات الى خمسة مجاميع كل مجموعة تحتوي على ٥ حيوانات وكما يلي :- فئران غير مصابة بالسكري (مجموعة السيطرة) ، فئران غير معالجة مصابة بداء السكري باستخدام الالوكسان ، فئران مستحدث فيها داء السكري معالجة بمستخلص المردقوش . حقنت الفئران المستحدث فيها داء السكري بالالوكزان بمستخلص المردقوش لمدة ١ ، ٢ ، ٣ اسابيع وبجرعة ٠.١٨ ملغم / كلغم من وزن الجسم . أظهرت الفئران المعاملة بالالوكزان لاستحداث داء السكري زيادة معنوية في مستوى الكلوكوز في الدم ، وانخفاض في العدد الكلي والتفريقي لكريات الدم البيض والاضداد (IgG , IGA , IGM) والمتمم C₃,C₄) مقارنة مع مجموعة السيطرة ، بينما اظهرت نتائج الفئران المستحدث فيها داء السكري بالالوكسان والمعالجة بمستخلص المردقوش . انخفاض معنوي (P<0.05) في مستوى الكلوكوز في الدم ، وزيادة في العدد الكلي والتفريقي لكريات الدم البيض والاضداد (IgG , IGA , IGM) والمتمم C₃,C₄) مقارنة مع مجموعة الفئران المستحدث فيها داء السكري بالالوكزان الغير المعالجة .

Abstract

It is aimed to study effects of *origanum vulgare* on serum glucose, blood film (total and differential white blood cells and immunoglobulin (IgG,IgM,IgA and complements C3 , C4) in alloxane-induced diabetic mice. Animals were divided into five groups of 5 animals each as follows: Non-diabetic mice (control group), non-treated alloxane diabetic mice, alloxane diabetic mice treated with *origanum vulgare*. Alloxan diabetic mice were injected with *Origanum Vulgare* extract for 1,2,3 weeks at a dosage of 0.18 g/kg body weight. The mice treated with alloxan (150 mg/kg B.W) to induce diabetic were showed increased in blood glucose level (hyperglycemia) , decreased in total and differential WBCs and immunoglobulin (IgG,IgM,IgA and complements C3 , C4) compared with control group, while the

results of alloxan diabetic mice treated with *origanum vulgare* showed significantly ($P < 0.05$) decreased in blood glucose level, increased in total and differential WBCs and immunoglobulin (IgG, IgM, IgA and complements C3, C4) compared with alloxan diabetic mice group without treatment.

Introduction

Diabetes mellitus is a complex disorder that is characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both caused by impaired metabolism of glucose, lipids and protein [1]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs [2]. In diabetic rats, the utilization of impaired carbohydrate leads to accelerated lipolysis, resulting in hyperlipidemia [3,4]. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies [5-10]. Antihyperglycemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature [11,12], however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect [13].

Oregano can be used for many things other than just a kitchen herb, and its medicinal uses are well documented in both Chinese and Western medicine. Before looking at the medicinal uses, however, a quick review of the herb itself and the active ingredients, will provide some of the theoretical information needed to understand these uses [14].

Used by the Chinese in the treatment of gastric problems, jaundice, vomiting and fever, oregano also contains the monoterpene phenol known as thymol that has strong antiseptic properties, and its isomer, carvacrol, another antibiotic that acts synergistically with thymol to increase the antibacterial effect of both. The ingredients of oregano oil are very similar to those found in thyme, which is used medicinally for much the same type of conditions as oregano [15]. Oregano extracts have been used for centuries to treat a variety of conditions such as those above that can be caused by microbial action, and also other conditions relating to the digestive, respiratory and immune systems [16].

In this experimental study, the effect of *origanum vulgare* on serum glucose, blood film (total and differential white blood cells and immunoglobulin (IgG, IgM, IgA and complements C3, C4) in alloxane-induced diabetic mice.

Materials and methods

Plant material

Origanum were purchased from the local market.

Extraction of *Origanum* compound (Alcohol Extract):

(50g) of plant powder was extracted with ethanole (250 ml) by Soxhelt apparatus for 6 hours at 40-60°C, then the cooled solution was evaporated to dryness by rotary evaporator at 40°C and kept until used [17].

Animals

Fifty adult males of Swiss albino strain mice were obtained from animal house. 25 mice were used throughout this study, the age of the mice were in the range of 2.5 to 3 months, and the weight in the range 25-30 grams. The animals were housed in small plastic cages, which were cleaned weekly by washing with soap and tap water and sterilized with 70% ethyl alcohol during the period of the study. The room temperature was maintained at (24±2) °C, and the animals were exposed to 14 hours light program. Food of animal was pellet from local market.

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrated (5% w/v) in physiological saline at a dose of 150 mg/kg body weight in a volume of 0.1ml. The diabetic state was confirmed 48 hours after alloxan injection by weight loss [18], and hyperglycaemia [19]. There was 75% mortality in animals treated with alloxan. Surviving mice with a fasting blood glucose level higher than 200mg/dl were included in the study. Five groups consisting of five animals for each group were maintained as follows:

Experimental groups

Control group:-Normal mice injected with 0.1ml of physiological saline.

Group A:-Normal mice injected with alloxan 0.1ml to induce diabetic mice.

Group B:-Diabetic mice treated with 0.1ml of *Origanum* extract after one week from treated with alloxan.

Group C:- Diabetic mice treated with 0.1ml of *Origanum* extract after two week from treated with alloxan.

Group D:- Diabetic mice treated with 0.1ml of *Origanum* extract after three week from treated with alloxan.

General procedure

Blood samples were collected by heart puncture for measured sugar levels at the end of (1,2 and 3 weeks) from treated with alloxan, the mice fasted over night and killed by cervical dislocation.

Blood film methods:

This methods was done according [20]

Differential count of leukocytes:

1- A small drops of heparinized blood which drawn from mouse was put on the end of clean, and dry slide. A pusher slide was place at an angle of 30° to 45° to the slide and then moved back to make contact with the drop. The forward movement of the pusher spreads the blood on the slide.

2-The blood film was allowed to dry in the air.

3-The slides were completely covered with Leishman stains, after 3 min., the slides were washed gently and then examined under light microscope and by applying the following equation :

$$\text{No. of cells (cells/mm}^3 \text{ blood)} = (\text{Total no. of leukocytes \%}) / 100.$$

Total count of leukocytes:

1-The blood was taken by heart puncher and put into heperinazed tube.

2-Adiluting solution (190µL) was pipetted into test tube.

3-The heperinazed blood (10µl) was pipetted and mixed well with diluting fluid for at least 2 minutes.

4-The hemocytometer was seted up with its cover glass in position and by a pasture pipette, both sides of the hemocytometer were filled with the diluted blood.

5- The cells were allowed for two minutes to be settled.

6-The cells were count in the four large squares on both sides of chamber using the 40 X objectives and subdued light.

7-The WBCs were calculated on the basis of cells counted, counted area, and the dilution.

$$\text{No. of cells (cells/mm}^3 \text{ blood)} = \text{No. of cells in four square} \times \text{correct volume} \times \text{correct dilution}/4.$$

Immunoassay kit (immunoglobulin)

1 -Open the plate, if moisture is present, allow to evaporate

2-Apply 5 pI of sample or control use human plasma as sample. Do not use heparin as anticoagulant.

3-Close the lid firmly. Incubate the plate at room temperature if possible inverted

4-Measure the diametr accurately to within 0,1 mm with a suitable device

5-Evaluate result using the table of refrence or standard curve

Biomaghreb.

Statistical analysis

Statistical analysis was performed to compare two different groups by using ANOVA-test. Statistical significance was determined at (P<0.05) [21].

Result and Discussion

The present study shows that mice given alloxane to induce diabetes show elevated blood glucose levels, (hyperglycemia) compared with the non-diabetic mice (table1). The physiological effects of alloxane, which kills the pancreatic cells and induces diabetes in dogs [22].

Table (1):- Serum glucose levels in control and alloxan diabetic mice

Groups		Glucose mg/dl (mean +SE)
Control		a 163.41+2.84
A Treated with alloxan		b 196.50+3.06
Treated with 0.1 ml of origanum extract after different period of alloxan treatment	B 1 week	c 186.41+2.03
	C 2 weeks	c 174.82+3.71
	D 3 weeks	a 168.84+2.55

Different letters means significant difference ($P<0.05$) (in rows)

Alloxan induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic β -cell, resulting in a decrease in endogenous insulin release[23,24].

Alloxane cause diabetes, the uric acid derivative initiates free radical damage to DNA in the beta cells of the pancreas, causing the cells to malfunction and die. When these beta cells fail to operate normally, they no longer produce enough insulin, or in other words, they cause one variety of adult-onset type 2 diabetes [25].

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and insufficiency of secretion or action of endogenous insulin. While exogenous insulin and other medications can control many aspects of diabetes, numerous complications affecting the vascular system, kidney, retina, lens, peripheral nerves, and skin are common and extremely costly in terms of longevity and quality of life [26]. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes mellitus and its complications [27]. Diabetes is usually accompanied by increased production of free radicals [28] or impaired antioxidant defences [29]. Under normal conditions, the body's natural defence enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), make free radicals innocuous by reducing superoxide radicals and peroxides, concurrently oxidizing glutathione [30]. While

hyperglycaemia engenders free radicals, on the other hand it also impairs the endogenous antioxidant defence system in many ways during diabetes [31]. Antioxidant defence mechanisms involve both enzymatic and non-enzymatic strategies. Liver and kidney are essential tissues where important complications of diabetes mellitus occur. It was shown that the severity of diabetic complications in tissues is related to the damage in their oxidative-antioxidative systems [32]. The results showed significant decreased in total and differential white blood cells (table 2) and immunoglobulin (IgG,IgM,IgA and complements C3 , C4) in alloxane-induced diabetic mice (table 3).

Table (2): Effects of organum extract on differential and total counts of blood in control and alloxan diabetic mice

Groups		IgG mg/dl (mean +SE)	IgM mg/dl (mean +SE)	IgA mg/dl (mean +SE)	C3mg/dl (mean +SE)	C4 mg/dl (mean +SE)
Control		A 1090.4+90.54	A 133.8+23.7	A 228.41+16.4	A 228.2+19.19	A 234.8+33.9
A Treated with alloxan		B 719.8+83.21	B 104.9+18.82	B 178.32+21.12	B 169.5+26.43	B 177.98+19.0
Treated with 0.1 ml of Origanum extract after different period of alloxan treatment	B 1 week	C 986.3+60.1	A 128.4+58.5	A 238.2+19.52	A 210.2+41.52	A 226.76+21.3
	C 2 weeks	C 1233.7+43.4	C 179.7+41.32	C 184.9+33.41	C 261.8+19.48	C 278.90+18.85
	D 3 weeks	D 1506.6+53.81	D 195.8+22.32	C 392.5+52.67	C 274.4+28.73	D 296.78+34.96

Different letters means significant difference (P<0.05) (in rows)

Table (3):- Immunoglobulin in control and alloxan diabetic mice

Groups		Total WBCs (m±SE) %	Differential count (m±SE)				
			No.of Lymphocyte cells /cu. mm. blood	No.of Neutrophil cells /cu. mm. blood	No.of Monocyte cells /cu. mm. blood	No.of Eosinophil cells /cu. mm. blood	No.of Basophil cells /cu. mm. blood
control		a 106.62+4.06	a 5720+636.2	a 3055+126.9	a 1612+131.4	a 40+20.3	a 0.00±0.00
A Treated with alloxan		b 86.40+1.89	b 2380+122.5	b 1961+86.4	b 753.4+62.2	b 122.0+56.0	b 320.18+81.4
Treated with 0.1 ml of origanum extract after different period of alloxan treatment	B 1 week	b 87.73+2.51	c 4460+212.4	c 2674+163.0	a 918.6+124.3	a 0.00±0.00	a 0.00±0.00
	C 2 weeks	c 94.42+2.93	c 5100+210.6	a 3433+78.6	a 1523+48.5	a 0.00±0.00	a 0.00±0.00
	D 3 weeks	a 102.34+2.61	a 6040+176.8	a 3688+76.5	a 1786.3+141.2	a 0.00±0.00	a 0.00±0.00

Different letters means significant difference (P<0.05) (in rows)

Macrophage lectin receptors that recognize bacterial cell wall sugars are reduced in alloxan diabetic mice. This is in contrast to the expression of macrophage Fc (IgG2b) receptors which remains unaltered. Peritoneal macrophages, from diabetic and normal mice, were used as a source of accessory cells in an antigen dependent T cell proliferation assay with unopsonized Staph. epidermidis as the antigen. Uptake of this antigen in the absence of serum is via the macrophage lectin receptors. We have shown that diabetic macrophages induce a level of antigen dependent T cell proliferation to Staph. epidermidis. However the T cell response to Con A was similar with both normal and diabetic macrophages. We suggest that the observed defect in antigen presentation by diabetic macrophages is at the level of uptake of antigen. High glucose levels, such as those found in diabetes, down-regulate the lectin receptor, reduce phagocytosis of Staph. epidermidis and affect antigen presentation. This has important consequences in terms of the ability of diabetics to mount an effective immune response [33].

The alloxane diabetic mice when treated with *origanum vulgare* showed significant increased in blood glucose, numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in alloxan-induced diabetic animals[34,35]. In the present study, the *origanum vulgare* extracts effectively decreased the blood glucose in alloxan-induced diabetic mice. The mechanism of these plants used has not been clearly defined. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver cell damage [36]. The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels and secondarily due to the effects of the diabetogenic agent alloxan[37]. *Origanum vulgare* contains chlorogenic acid and its related compounds, 3,5-dicaffeoylquinic acid (SP-1) and 4- succinyl-3,5-dicaffeoylquinic acid (SP-2), known as its major antioxidants[38]. In our previous study, *origanum vulgare* extract showed strong free radical scavenging and antioxidant activities and also showed a protective effect on DNA damage caused by hydroxyl radicals[39]. Based on above-mentioned reports, we suggest that the possible mechanism of action by aqueous extracts from *origanum vulgare* could be related to antioxidants that aid to recover from impaired metabolism of glucose. Previous studies have demonstrated that *D. batatas* may prove helpful as a digestion-aiding agent for patients suffering from hyperglycemia[40] or hyperlipidemia[41], but we have experienced a negative result regarding the hypoglycemic effect.

The results suggest that diabetes may increase oxidative stress in liver and kidney tissues and *origanum vulgare* might have a protective affect via them free radical-scavenging properties in these tissues.

Origanum vulgare extract increased total and differential white blood cells and immunoglobulin (IgG,IgM,IgA and complements C3 , C4) in alloxane-induced diabetic mice.

All these effects on total and differential counts of leukocyte by *Origanum vulgare* extract have one explanation in which *Origanum vulgare* extract have no

cytotoxic effect this is due to most active compounds which have medicinal activity [42], like glycosides , flavonoids especially rutin and quercetin which have an important roles in immune system as an immune stimulators [43] , and this increases was cleared in WBCs especially lymphocyte which acts as a regular of immune response through it's secretion to cytokines which act as activator for immune system through induces to phagocytes and the induction of B-cell to produce antibody . [44] , in addition to the increases in number of monocytes have an important role in phagocytosis [45].

Its effect on respiratory problems is also likely due to the antibacterial action of the thymol and carvacrol. Oregano extract has also been found effective in treating toothache, earache and relieving the itch of insect bites. It contains a number of minerals, such as manganese, copper, iron, zinc, potassium and calcium, essential in human biochemistry, and vitamins A and C that are strong antioxidants. The effect of oregano in helping to support the immune system through its antioxidant effect is also likely to be a significant factor in its curative properties for so many diseases and infections [46].

Comfrey, one of the most valuable herbs known to botanical medicine, has been used for centuries to heal. It is full of amino acid, lysine, B12, and vitamins A and C as well as high in calcium, potassium, phosphorus, and protein. Additionally, it contains iron, magnesium, sulphur, copper, zinc, and eighteen amino acids. Echinacea is an herb that stimulates immunity within the body and increases its ability to fight infections. It includes vitamins A, E, and C, as well as iron, iodine, copper,...[47].

Echinacea, zinc, vitamin C, and garlic are all the most well-known and respected supplements concerning the maintenance and strengthening of the immune system. Echinacea contains polysaccharides, flavonoids, caffeic acid derivatives, essential oils, polyacetylenes, and alkylamides, which contribute to the herb's therapeutic benefits. These constituents are responsible for a variety of immuno-stimulatory, anti-inflammatory, antiviral, antibacterial, and anticancer properties. Echinacea...[48].

Studies show that you can reverse the effects of alloxan by supplementing your diet with vitamin E. Natural Healing, vitamin E effectively protected lab rats from the harmful effects of administered alloxan. Now, you're not a lab rat, but you're a mammal and vitamin E is definitely worth adding to your daily regimen of nutritional supplements, especially if you have a history of eating foods made with white flour and are at high risk for diabetes .

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