IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2017 Vol.:8, Issue:3

Effect of the Combination of Fenugreek Seeds Oil and Olive Leaves Extracts on Diabetic (II) Albino Mice

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ISSN 2349-7203
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Submission: 2 February 2017
Accepted: 7 February 2017
Published: 25 February 2017

Keywords: olives leave extract, fenugreek seed oil, diabetic Albino mice, phytochemical analysis.

ABSTRACT

The aim of present study was to determine the effects of crude aqueous extract of *olive leaves*, *fenugreek seeds* oil and their mixture on alloxan diabetic mice, (84) adult male albino mice were divided randomly into (6) groups, each group included (8) mice, the first group (G_1) served as control group, the second group (G_2) served as alloxan induced diabetic mice, the third, fourth, fifth groups were treated with the extract of *olive leave*, *fenugreek seed* oil and their mixture, dosage (50, 100, 200) mg/kg of animal weight daily for (4) weeks. The results showed significant decrease of blood sugar (b. s) levels from (285± 8.1) mg/dl to (165± 7.2) mg/dl using *olive leaves* extract (200mg/ Kg. b. w), also the result was decreased from (289± 8.9) mg/dl to (170± 7.2) mg/dl when used *fenugreek* seeds oil (200 mg/kg. b. w), while the result was showed clear significant differences in reduction of the blood sugar level from (289± 8.9) mg/dl to (154± 7.7) mg/dl when using combination of crude *olive leaves* extract and *fenugreek seeds* oil (200 mg/kg. b. w). All the types of crude *olive leaves* extract and fenugreek seeds oil contains a number of medicinally important compounds, that were indicated by phytochemical analysis in different amounts such as tannins, carbohydrate, glycosides, resins, flavonoids, saponin, alkaloid and phenols.

1. INTRODUCTION

Plants are used medicinally in different countries are a source of many potent and powerful drugs. Fenugreek is a medicinal plant that uses in disease some therapy. Natural products have been a major source of new drugs [1]. Medicinal plants are used 80% of the world population as the only available medicines especially in developing countries [2]. Fenugreek is one of the oldest medicinal plants [3], use for blood lipids and sugar decreasing in diabetic and nondiabetic peoples and have antioxidant and antibacterial activity [4,5]. Fenugreek seed powder in the diet reduces blood sugar and urine sugar with concomitant improvement in glucose tolerance and diabetic symptoms in type II diabetic patients [6]. The hypoglycemic effects of fenugreek have been attributed to several mechanisms, demonstrated in vitro the amino acid 4-hydroxyisoleucine in fenugreek seeds increased glucose-induced insulin release in human and rat pancreatic islet cells, it was observed that 4-hydroxyisoleucine extracted from fenugreek seeds has insulin tropic activity [7]. Fenugreek seeds contain 50% fiber (30% soluble fiber and 20% insoluble fiber) that can slow the rate of postprandial glucose absorption [8].

The olive (*Olea europaea*) belongs to the family Oleaceae, species of a small tree. The olive tree is an evergreen tree or native to the Mediterranean shrub, Asia and Africa [9]. Olive leaves are source of many phytochemicals like phenolics and flavonoids which possess many activities e.g. antioxidant, antibacterial, antifungal etc. [10]. Oleuropein is the major phenolic compound in olive leaves and varies from 17% to 23% depending on the harvesting time of the leaves [11]. Olive leaves have an ethnomedical usage in the management of diabetes, and the anti-diabetic effect of olive leaves or oleuropein has already been demonstrated in animal models [12,13].

The aim of this study was to determine the effects of crude aqueous extract of *olive leaves*, *fenugreek seeds* oil and their mixture on alloxan diabetic mice.

2. FURTHER INFORMATION

2.1. *Olive leaves* and *fenugreek seeds*, collection:

The *olive leaves* were purchased from local garden around the status of research center in Baghdad, Iraq as dark green leaves, while *fenugreek seeds* purchased from local herb store in Baghdad as pale brown, both herb plants used in present study, classified by botany specialist.

2.2. Preparation of aqueous extract of *olive leaves*:

(300) grams powder of *olive leaves* macerated with (1000) ml of water using shaker device (SI-600R) for (6) hr. at (90) ˚C. The extract was filtrated then drying using (BÜCHI Mini Spray Dryer B-290) aqueous extract was obtained.

2.3. Preparation of *fenugreek seeds* oil:

(100) grams powder of *fenugreek seeds* was extracted with (750) ml of hexane at (70)˚C for (10) hours using soxhlet apparatus. The extract was concentrated under reduced pressure in rotary evaporator at (70)˚C to remove all solvent *fenugreek seeds* oil was obtained.

2.4. Phytochemical analysis:

Phytochemical analyses of the crude *olive leaves* extract and *fenugreek seeds* oil that obtained by different methods [14], indicated different amount of tannins, carbohydrate, glycosides, resins, flavonoids, saponin, alkaloid and phenols.

2.5. Dosage by alloxan:

For expand the level of (b.s) in laboratory mice by dosage fasting animals for (24) hours with (150) mg from alloxan/kg from the weight of animal in peritoneal membrane for one time and dosage animal with (1) ml from (10%) of (b. s) solution to rapidly happen of diabetes, then measurement the level of (b. s) in next days until ensure that the value of sugar is more than (250) mg/100 ml of blood [15].

2.6. Design the animal experiment:

This experiment had been designed on (3) stages, used (84) mice (male), which bought from pharmaceutical and biological control center in ministry of health. The mice were dividing into (6) groups and lifted for (2) weeks. First group considered as control, while the second- third- fourth- fifth and sixth groups dosage with (150) mg/ kg of alloxan, to increase the level of sugar more than (250) mg/(100) ml in the blood. Dosage the animal (third, fourth, fifth groups respectively) continues one time for (4) weeks with (200) ml of *olive leaves* extract with different concentration (50,100,200) mg/kg of animal weight and the sixth group dosage with (200) ml which contain (600) mg of kalbinamide (chemical drug) that uses for treatment the diabetes type II and considered as a positive group, while first group (control) dosage with same amount of distilled water. Period the measured of the (b. s) levels, weight of animal and consumption rate of water. After finished this experiments, all mice were sacrificed and collected the blood to complete other chemical tests, while tissues such as liver and pancreas

placed in (10%) formalin to making tissue analysis. This experiment had been replicated with same design on *fenugreek seeds* oil and the mixture of both *olive leaves* extract, *fenugreek seeds* oil to define their activity on the (b. s) levels [16].

2.7. Statistical analysis:

The statistical analyses were performed using the SPSS Ver. (19) program (Systat Software Inc., Chicago, IL, USA). Values were compared to control using analysis of variance (ANOVA) followed by Duncan's post hoc test. P values < 0.05 were considered significant.

3. RESULT AND DISCUSSION

3.1. Effect of crude aqueous extract of *olive leaves*, *fenugreek seeds* oil and their mixture on (b. s) levels on alloxan diabetic mice**:**

The treatment of diabetes with synthetic drugs is costly and chances of side effects are high, medicinal plants and phytoconstituents play an important role in the management of diabetes mellitus especially in developing countries [17], therefore in this work studied the effect of crude aqueous extract of *olive leaves*, *fenugreek seeds* oil and their mixture on the (b. s) levels of laboratory diabetic mice, the results showed decreasing of the (b. s) levels after (4) weeks from the zero time due to the present of a different amount of phytochemical groups that effect in the activity of crude aqueous extract of *olive leaves*, *fenugreek seeds* oil and their mixture such as tannins, carbohydrate, glycosides, resins, flavonoids, saponin, alkaloid and phenols which were responsible for anti-diabetics effects, tables (1), (2) and (3).

Table (1): The effect of crude aqueous extract of *olive leaves* on diabetic mice.

Parameter	Time (wk)	Control (G1)	Alloxan (G2)	50 mg/kg (G3)	100 mg/kg (G4)	200 mg/kg (G5)	600µg/kg drug (G6)
Body weight (gm)	0	22±0.20	24±0.32	24±0.29	25±0.33	25±0.32	23±0.22
	2	24±0.17	21±0.21	22±0.21	23±0.24	24±0.24	24±0.19
	4	23±0.18	19±0.24	20±0.21	21±0.22	24±0.20**	23±0.27
Fluid intake ml/day	0	4.1±2.5	4.3±2.4	4.1±7.2	4.0±2.6	4.3±1.5	4.1±2.7
	2	4.2±1.8	5.8±2.1	5.6±1.8	5.0±2.3	4.7±2.9*	4.2±1.9
	4	4.3±1.5	6.8±4.6	4.9±1.5	4.5±4.4*	4.4±4.3**	4.3±1.7
B. sugar mg/dl	0	88±4.5	278±7.8	280±8.1	288±9.2	285±8.1	175±8.1
	2	92±3.8	290±9.5	230±7.9	212±8.6*	180±7.1**	160±7.1
	4	94±6.2	350±9.9	189±10*	177±4.6**	165±7.7***	140±7.7

The percentage of (b. s) levels decrease comparing with healthy mice (control), appear significant differences in value of (P‹0.05).

Table (2): The effect of *fenugreek* seed oil on diabetic mice.

Parameter	Time (wk)	Control (G1)	Alloxan (G2)	50 mg/kg (G3)	100 mg/kg (G4)	200 mg/kg (G5)	600µg/kg drug (G6)
Body weight (gm)	0	23±0.21	24±0.18	23±0.27	23+0.30	23±0.32	23±0.21
	2	22±0.18	21±0.22	21±0.24	22±0.24	23±0.24	24±0.18
	4	24±0.19	19±0.23	20±0.22	21±0.22	22±0.23	23±0.25
Fluid intake ml/day	0	4.1±2.5	4.3±2.4	4.2±2.3	4.2±2.5	4.3±2.2	4.1±2.6
	2	4.2±1.8	5.8±2.1	5.7±1.5	5.2±2.6	4.7±2.7	4.4 ±2.1
	4	4.3±1.5	6.8±4.6	4.9±1.8	4.5±4.4	4.4±4.3	4.3±2.6
B. sugar mg/dl	0	88±4.4	288±9.7	282±8.9	279±9.0	289±8.9	285±8.7
	2	90±4.5	350±10	240±7.7	219±8.2*	200±9.3*	164±6.9
	4	94±5.2	370±9.9	200±9.6	180±4.3**	170±7.2***	140±8.9

The percentage of (b. s) levels decrease comparing with healthy mice (control), appear significant differences in value of (P‹0.05).

Table (3): The effect of the combination of crude aqueous extract of *olive* leaves and *fenugreek* seeds Oil on diabetic mice.

Parameter	Time (wk)	Control (G1)	Alloxan (G2)	50 mg/kg (G3)	100 mg/kg (G4)	200 mg/kg (G5)	600µg/kg drug (G6)
Body weight (gm)	0	22±0.20	24±0.32	24±0.29	25+0.33	23±0.32	23±0.22
	2	24±0.17	21±0.21	22±0.21	23±0.24	24±0.24	24±0.19
	4	23±0.18	19±0.24	20±0.24	21±0.22	23±0.20	23±0.27
Fluid intake ml/day	0	4.1±2.5	4.3±2.4	4.1±7.2	4.2±2.6	4.3±1.5	4.1±2.7
	2	4.2±1.8	5.8±2.1	5.6±1.8	5.0±2.3	4.7±2.9	4.2 ±1.9
	4	4.3±1.5	6.8±4.6	4.9±1.5	4.5±4.4	4.4±4.3	4.3±1.7
B. sugar mg/dl	0	88±4.4	288±9.7	282±8.9	279±9.0	289±8.9	285±8.7
	2	92±3.8	290±9.5	200±7.9*	177±8.6*	169±7.1**	160±7.1
	4	94±6.2	350±9.9	184±10*	160±4.6**	154±7.7***	140±7.7

The percentage of (b. s) levels decrease comparing with healthy mice (control), appear significant differences in value of ($P<0.05$).

3.2. Histological study:

Animals of the control group did not appear to have any histological changes during the stages of experiment since all the islets of Langerhans of the control animals appeared regular in shape with no marked differences between them, small islets of about (21µ) in diameter and reached (38µ) in large islets. Islets of healthy non-diabetic animals had well defined boundaries. Most of the cells were of the β-type. B-cells were small polygonal arranged in groups & cords fine capillaries as showed in figure (1).

3.3. Diabetic islets:

Histological sections from endocrine regions of pancreatic tissue of alloxan-induced diabetic mice revealed shrinkage of β-cells of islets of Langerhans and a significant reduction in the size of the islets when compared to that of normal groups. Pancreatic sections stained with hematoxylin and eosin (H&E) showed that alloxan caused severe necrotic changes of pancreatic islets, especially in the center of islets. Nuclear changes, karyolysis, disappearance of nucleus and in some places, residue of destroyed cells were visible. Relative reduction of size and number of islets especially around the central vessel and severe reduction of beta cells were clear as shown in figure (2). Further, the study revealed the presence of damaged β-cell population. This damage of the β-cells due to alloxan induction [18].

The possible mechanism for β-cell destruction by alloxan has been reported to include generation of some types of oxygen free radicals and alternation of endogenous scavengers of these reactive species. It has been suggested that reactive oxygen species are a contributory factor in the development of diabetes complications. There are many reports indicating changes in the parameters of oxidative stress in diabetes mellitus. Among the antioxidant defense mechanisms are Glutathione (GSH) and uric acid that remove reactive oxygen species once formed. These observations are in accordance with the findings that alloxan results in hepatic (GSH) content depletion in due to the higher level of free radical generation that convert more reduced (GSH) to its oxidized form. In diabetes, the increased (b. s) levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from beta cells manifest in the decreased serum insulin levels. The reduction in the serum insulin levels in the alloxan-treated might be attributed to the reduced secretion of the hormone which might be due to the

damage of the beta cells of endocrine pancreas. The alloxan selectively destroys the pancreatic cells and induce hyperglycemia.

Study of pancreas of treated diabetic groups showed increased size of islets and hyperchromic nucleus in sections stained with H & E. These were also a relative increase of granulated and normal beta cells in the diabetic group which consumed the extract and the clear effect was depending on dose of extract administrated, so 200 mg/kg body weight of extract have the most effect on regeneration of beta cell as seen in figure (3). The histopathological study of diabetic treated group indicated increased volume density of islets and increased percentage of beta cells, in the diabetics that received the extracts, which may be a sign of regeneration. Signs of regeneration of ß cells, potentiation of insulin secretion from surviving ß cells of the islets of Langerhans and decrease of (b. s) that have been reported following consumption of some plant extracts.

The results showed rehabilitation and activation of shattered beta cells by dosage mice with alloxan which expands secretion of insulin and decreases sugar, tissues of Langerhans were showed normal in control after (4) weeks and not appear any pathological changes, figure (1). The outer tissues of pancreas secretions consist of acini and cannulae, founded the components of endocrine which secreted many of hormones such as insulin which secret from Langerhans and metabolism of sugar and carbohydrates, misalignment of these endocrine lead to diabetes, in the figures of Langerhans of pancreas in mice which induced with diabetes creator by alloxan and non-treated with any extract showed changes included destroyed of beta cells and distortion of cells, the changes in Langerhans indicate the occurrence of fibrosis, figure (2). While showed in figure (3) remarkable improvement in amount of beta cells which indicate rehabilitation and restoration of beta cells in mice which treated with the combination of crude aqueous of *olive* leaves extract and *fenugreek* seeds oil, so decreased (b. s) level in this group comparing with control group.

On the other hand, studies on the supplementation of extracts the diabetic revealed restoration of size of the islets along with β-cells repair. This recovery of the β-cells was recorded as dose (300) mg /kg body weight of the extract given animals. The extract fed animals revealed better-restored β-cells of pancreas from the alloxan-induced damage, the major bioactive compounds of the combination of crude aqueous of *olive* leaves extract and *fenugreek* seeds oil, were found to be flavonoids alkaloids, phenolic (oleuropein) compounds [19,20,21,22,23]; the main function of these compounds are antioxidant activity. Supplementation of antioxidants may be a protective

factor against free radical induced beta cell damage, thus preventing or ameliorating diabetes mellitus. On the other hand, the major bioactive compounds may be inhibiting c-AMP phosphodiesterase and c-AMP is a modulator of insulin secretion.

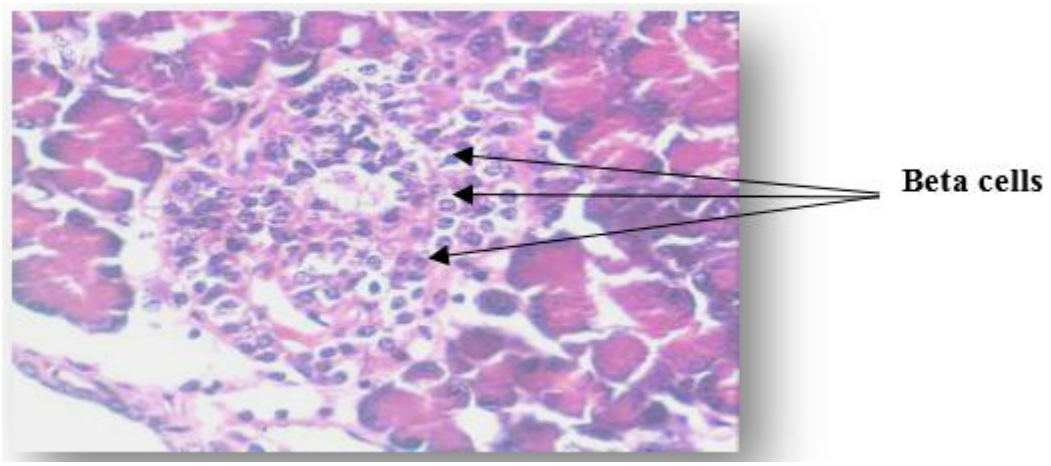

Figure (1): Photomicrograph of pancreas tissue for healthy non-diabetic mice stained with hematoxylin and Eosin (Magnification power x 200) showing normal cells in the islet of Langerhans.

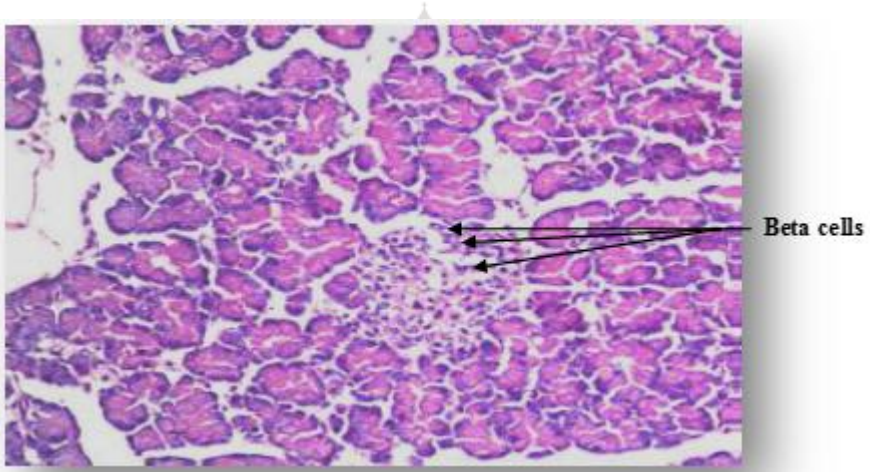

Figure (2): Photomicrograph of pancreatic tissue from alloxan-induced diabetic mice stained with hematoxylin and Eosin (magnification×200), showing advanced changes of diabetes as destruction of beta cells with pyknosis of nuclei. Observe distortion of cells and reticular changes of islets as evidence of fibrosis

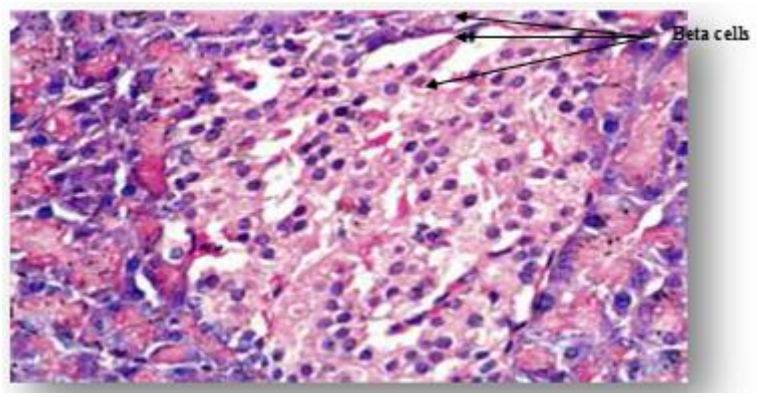

Figure (3): Photomicrograph of pancreatic tissue from alloxan-induced diabetic mice treated with 200 mg/kg. b. w of hexane extract stained with hematoxylin and Eosin (magnification×200), showing more improvement and generation of new cellular population size of islets (H&E) (10X20).

4. CONCLUSION

This study showed clearly the different effects of the extracts on the (b. s) levels in the diabetic albino mice that created by alloxan. This was confirmed with tissue experiments for pancreas of the albino mice, which treated by using extracts, comparing to the positive control (treatment with klbenamide) and negative control (second group which induced with diabetic and was not treated with any drug).

5. ACKNOWLEDGMENT

The Author thanks, Dr. Hassan Fayadh Al'Azzawi, Department of Biotechnology, College of Science, Baghdad University, Al-Jadriyah, Baghdad, Iraq for his advice.

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