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# Comparison of (*Boswellia sp.*) Extracts by Different Methods on Diabetic Albino Mice

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**ABSTRACT**

The aim of this study is to evaluate the effect of crude extracts of *Boswellia sp.* using different traditional methods (continuous reflux extraction, maceration) on alloxan diabetic mice, (90) adult male albino mice were divided randomly into (6) groups, every group included (8) mice, the first group (G1) served as control group, the second group (G2) served as alloxan induced diabetic mice, the third, fourth, fifth groups were treated with extracts of *Boswellia sp*., 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± 8.6) mg/dl to (209± 9.7) mg/dl using cold aqueous extract (200mg/ Kg. b. w), while the result was similar when used hot aqueous extract (200mg/ Kg. b. w) as the cold aqueous, also the result was showed clear significant differences in reduction of the (b.s) level from (289± 9.9) mg/dl to (172± 7.2) mg/dl when using methanol extract, the hexane extract (200mg/Kg. b. w) showed considering statically differences in reduction of the (b.s) level from (289± 8.9)mg/dl to (170±8.3)mg/ dl. All the types of crude extracts of *Boswellia sp.* contain a number of medicinally important compounds, that were indicated by phytochemical analysis in different amount such as tannins, carbohydrate, glycosides, resins, flavonoids, saponin, alkaloid and terpenes.

## 1. INTRODUCTION

In the recent years, there has been a tendency to use of medicinal plants because of the lower side effects and variety of effective compounds in plants[1]. Type (II) diabetes is one of the most serious endocrine disorders in worldwide [2]. It has predicted that the diabetes prevalence among the people, would reach to (552) million populations in year (2030) [3]. *Boswellia* subspecies are trees (family: Burseraceae), found in India, Northern Africa and the Middle East [4]. Frankincense is the hardened gum resin extruded from incisions in the trunk of several *Boswellia* species[5]. The genus *Boswellia* has approximately (20) species occurring in the dry regions spanning from West Africa to Arabia and south to the northeast region of Tanzania [6]. Members of this genus are trees or shrubs that are described as having outer barks that peel in parchment flakes, a greenish inner bark, watery aromatic resins, and wood with a milky latex [7]. Frankincense, or olibanum, is the oleogum resin that is harvested from several different trees belonging to the genus *Boswellia.* The word frankincense is deriving from the ancient French name "frankincense", meaning "pure incense". Also known in Arabic as "luban", which means "white" or "cream": in Greek as "libanos"; in Ethiopia as "etan"[8,9,10,11,12,13,14]. *Boswellia* resin is a mixture containing more than (200) different substance [15], for instance: resin, long chain sugar compounds, essential oils, proteins, *β*-boswellic acid, 3-*0*-acetyl-11-keto-β-boswellic acid[16, 17]. The quality and commercial value of resins differ based on the species from which they are obtaining [18].

The aim of this study is to evaluate the effect of crude extracts of *Boswellia sp*., using different solvents and traditional methods on alloxan diabetic mice.

## 2. MATERIALS AND METHODS

2.1.*Boswellia sp.* collection:

The medicinal plant used in this study purchased from local herb store in Baghdad, Iraq as yellow- pale brown granules and classified by botany specialist.

2.2. Preparations of cold aqueous and Methanol extracts have same procedure but using different solvents as follow:

(300) ml of solvent was sonicated with (100) grams powder of *Boswellia sp*. using Ultrasonic device for (6) hours. The extract was filtrated then it was concentrated under reduced pressure using rotary evaporator at (70)˚C to remove all the solvent. The extract was drying using Oven under Vacuum at (40)˚C. (7.3) grams of cold aqueous extract and (5.4) grams of methanolic extract were obtained.

Preparations of hot aqueous and hexane extracts have same procedure but using different solvents as follow:

(100) grams powder of *Boswellia sp*. was extracted with (1L) of solvent at (70)˚C for 10 hours in soxhlet apparatus. The extract was concentrated under reduced pressure in a rotary evaporator at (70)˚C to remove all the solvent, then drying using Oven under Vacuum at (40)˚C. Net weight was obtained (6.1, 5.5) grams for hot aqueous and hexane extracts [19].

2.3. Phytochemical analysis:

Phytochemical analyses of four crude extracts *Boswellia sp*. that was obtained by different methods[20], indicated different amount of tannins, carbohydrate, glycosides, resins, flavonoids, saponin, alkaloid and terpenes.

2.4. 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 glucose solution for rapid incidence 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 [21].

2.5. Design the animal experiment:

This experiment had been designed on (3) stages, used (90) 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 *Boswellia sp.* 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 of the measured the (b.s) levels, weight of animal and consumption rate of water. After finished this experiment, 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 make tissue analysis. This experiment had been replicated with same design on other extracts such as hot aqueous extract, alcoholic (methanol) and hexane to define the activity of these extracts on the (b.s) levels [22].

2.6. 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. RESULTS AND DISCUSSION

3.1. Effect of extracts:

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 [23], therefore in this work using *Boswellia sp*. extracts and study their effect with different solvents (hot & cold aqueous, methanol and hexane) 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 *Boswellia sp*. extracts such as tannins, carbohydrate, glycosides, flavonoids, alkaloid and terpenes which were responsible for anti-diabetics effects, tables (1), (2), (3) and (4).

**Table (1): The effect of cold crude aqueous extract of *Boswellia sp.* on diabetic mice.**

| Parameter | Time (wk) | Control (G1) | Alloxan (G2) | 50 mg/kg (G3) | 100 mg/kg (G4) | 200 mg/kg (G5) | 600 mg/kg drug (G6) |
|---|---|---|---|---|---|---|---|
| Body weight (gm) | 0 | 23±o.26 | 24±o.26 | 24±0.29 | 25+0.33 | 23±0.32 | 23±0.22 |
| | 2 | 23±o.27 | 21± 0.20 | 22±0.21 | 23±0.24 | 24±0.24 | 24±0.19 |
| | 4 | 23±o.19 | 19± 0.21 | 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 | 4.0±2.6 | 403±1.5 | 4.1±207 |
| | 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± 6.5 | 288±9.8 | 286±9.4 | 288±9.5 | 285±8.6 | 279±8.7 |
| | 2 | 92 ±4.8 | 295±9.9 | 245±7.6 | 231±8.7 | 218±7.7 | 170±7.1 |
| | 4 | 94± 6.7 | 350±9.2 | 228±8.2 | 218±8.3 | 209±7.6 | 140±7.7 |

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

**Table (2): The effect of hot crud aqueous extract of *Boswellia Sp.* on diabetic mice.**

| Parameter | Time (wk) | Control (G1) | Alloxan (G2) | 50 mg/kg (G3) | 100 mg/kg (G4) | 200 mg/kg (G5) | 600 mg/kg drug (G6) |
|---|---|---|---|---|---|---|---|
| Body weight (gm) | 0 | 23±o.26 | 24±o.26 | 24±0.29 | 25+0.33 | 23±0.32 | 23±0.22 |
| | 2 | 23±o.27 | 21± 0.20 | 22±0.21 | 23±0.24 | 24±0.24 | 24±0.19 |
| | 4 | 23±o.19 | 19± 0.21 | 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 | 4.0±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± 6.5 | 288±9.8 | 286±9.2 | 288±9.7 | 285±8.4 | 279±8.7 |
| | 2 | 92 ±4.8 | 295±9.9 | 242±8.6 | 222±8.2 | 212±7.8 | 170±7.1 |
| | 4 | 94± 6.7 | 350±9.2 | 220±7.9 | 210±8.6 | 200±7.9 | 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 (3): The effect of methanol crude extract of *Boswellia sp.* on diabetic mice.**

| Parameter | Time (wk) | Control (G1) | Alloxan (G2) | 50 mg/kg (G3) | 100 mg/kg (G4) | 200 mg/kg (G5) | 600 mg/kg drug (G6) |
|---|---|---|---|---|---|---|---|
| Body weight (gm) | 0 | 23±o.26 | 24±o.26 | 24±0.29 | 25+0.33 | 23±0.32 | 23±0.22 |
| | 2 | 23±o.27 | 21± 0.20 | 22±0.21 | 23±0.24 | 24±0.24 | 24±0.19 |
| | 4 | 23±o.19 | 19± 0.21 | 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 | 4.0±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± 6.5 | 288±9.8 | 286±9.7 | 288±9.2 | 285±8.4 | 279±8.7 |
| | 2 | 92 ±4.8 | 295±9.9 | 232±8.3 | 208±7.9 | 192±6.8 | 170±7.1 |
| | 4 | 94± 6.7 | 350±9.2 | 194±7.7 | 185±6.6 | 172±7.9 | 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 (4): The effect of hexane extract of *Boswellia sp.* on diabetic mice.**

| Parameter | Time (wk) | Control (G1) | Alloxan (G2) | 50 mg/kg (G3) | 100 mg/kg (G4) | 200 mg/kg (G5) | 600 mg/kg drug (G6) |
|---|---|---|---|---|---|---|---|
| Body weight (gm) | 0 | 23±0.21 | 24±o.18 | 23±0.27 | 23+0.30 | 23±0.32 | 23±0.22 |
| | 2 | 22±0.18 | 21± 0.22 | 21±0.24 | 22±0.24 | 24±0.24 | 24±0.19 |
| | 4 | 24±o.19 | 19± 0.23 | 20±0.22 | 21±0.22 | 23±0.20 | 23±0.27 |
| Fluid Intake Ml/day | 0 | 4.1±2.5 | 4.3± 2.4 | 4.2±2.3 | 5.0±2.6 | 4.3±1.5 | 4.1±2.7 |
| | 2 | 4.2±1.8 | 5.8±2.1 | 5.7±1.5 | 5.0±2.3 | 4.7±2.9 | 4.2±1.9 |
| | 4 | 4.3±1.5 | 6.8±4.6 | 4.9±1.8 | 4.5±4.4 | 4.4±4.3 | 4.3±1.7 |
| B. Sugar Mg/ dl | 0 | 88± 4.4 | 288±9.8 | 282±8.9 | 288±9.2 | 285±8.4 | 279±8.7 |
| | 2 | 90 ±4.5 | 295±9.9 | 251±8.7 | 208±7.9 | 192±6.8 | 170±7.1 |
| | 4 | 94± 5.2 | 350±9.2 | 210±7.4 | 185±6.6 | 172±7.9 | 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 the 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 in pancreatic islets, especially in the centre 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 showed in figure (2). Further the study revealed the presence of damaged β-cell population. This damage of the β-cells due to alloxan induction.

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 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 converts more reduced (GSH) to its oxidized form. In diabetes, 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 200mg/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 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 expand secretion of insulin and decrease 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 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 extract of *Boswellia sp.*, 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 at dose (300) mg /kg body weight in 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 alcoholic and hexane extracts were found to be flavonoids compounds [24]; 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, flavonoids may be inhibiting c-AMP phosphodiesterase and c-AMP is a modulator of insulin secretion. So it could be concluded that the anti-diabetic effect of extracts were due to the isoterpenes compounds.

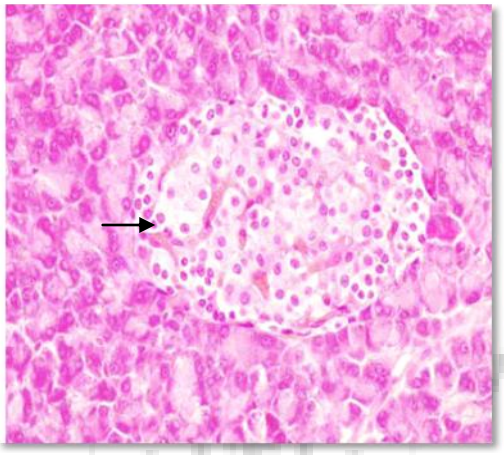

**Figure (1): Photomicrograph of pancreas tissue for healthy non diabetic mice stained with hematoxylin and Eosin (Magnification powerx 200) showing normal cells in the islet of langerhans.**

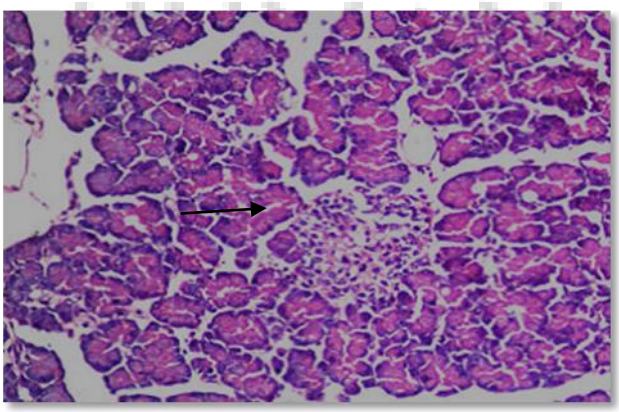

**Figure (2): Photomicrograph of pancreatic tissue from alloxan-induced diabetic mice stained with hematoxylin and Eosin (magnification×200), showing advanced changes of diabetes as destruction of beta cells with pyknosis of nuclei. Observe distortion of cells and reticular changes of islets as evidence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

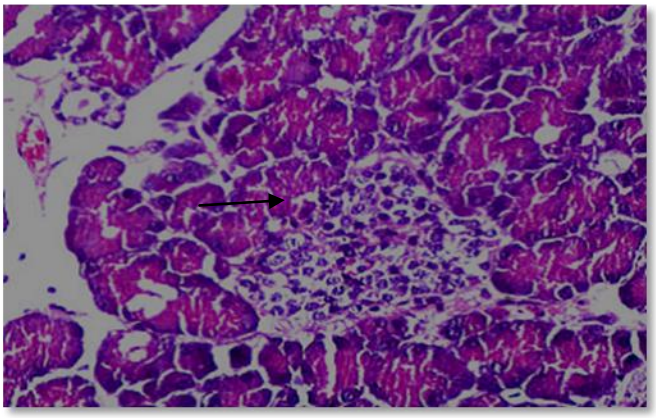

**Figure (3): Photomicrograph of pancreatic tissue from alloxan-induced diabetic mice treated with 200mg/kg. b.w of hexane extract stained with hematoxylin and Eosin (magnification×200), showing more improvement and generation of new cellular population size of islets (H&E) (10X20).**

## 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 effect can be attributed to the different solvent that used in extraction of *Boswellia sp.* with different methods. 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).

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