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Effect Of Boswellia Carterii Birdw On Carbohydrate Metabolism In Diabetic Male Albino Rats

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Abstract

In the current study, thirty male adult albino rats were used to investigate the effect of Boswellia Carterii Birdw (BCB) on carbohydrate metabolism in alloxan-induced diabetes. Rats were divided into three equal groups, control, diabetic non treated and diabetic BCB treated groups. After thirty days of treatment five rats of each group were sacrificed and the others were left without any additional treatment for another 15 days (recovery period) then were sacrificed. The body weight of each rat was determined at the beginning and the end of each period. Blood glucose, serum insulin and liver glycogen were determined for each rat at the end of each period. It was noticed that B.C.B treatment led to a significant improve in the decreased body weight, hyperglycemia, hypoinsulinemia, decreased liver glycogen caused by alloxan. And this improvement was also seen after the recovery period.

B.C.B treatment led also to marked improvement in the histopathological degenerative changes in the **β** cells of islets of Langerhans caused by alloxan after both the treated and recovery periods.

Introduction

The incidence of diabetes mellitus in the human population has reached epidemic proportions worldwide, and it is increasing at a rapid rate **(*Gannong, 2003*)**. Being a chronic disease it needs log term treatment either with insulin or the traditional oral hypoglycemic drugs and these cause financial exhaustion of the patients. Diabetic poor patients always search for a cheaper substitute in the medicinal plants.

Since ancient times diabetes mellitus has been treated orally with herbal remedies based on folk medicine. More than 400 traditional plant for treatment of diabetes have been recorded , but only few of them have received scientific and medical evaluation to assess their efficacy **(*Begman and Bari, 1985*)**.

Boswellia Carterii Birdw (BCB) is one of the most important medicinal plants which has been used for treatment of many diseases three hundred years ago ***(Zhang,2001)***. The plant resin was proved to have an anti-inflammatory effects so it is used in the treatment of ulcerative colitis ***(Gerhardt et al., 2001),*** chronic colitis ***(Keila et al., 2005)***, Crohn's disease ***(Gupta et al., 2001)***, polyartheritis ***(Sander, 1998)*** and osteoarthritis ***(Kimmatker et al., 2003)***. It also has apoptosis inducing effect and cytostatic effect , so it is used in treatment of acute non lymphocytic leukemia and tumors **(*Liu et al., 2003)***. In the folk medicine Boswellia Carterii Birdw is prescribed either alone or in combination with other plants for diabetic patients and many of them reported good benefit. The effect of BCB alone on diabetes has not been investigated previously, so this work is a trial to investigated the relation between BCB and treatment of diabetes mellitus.

Material And Methods

Material:

A-Animals: Thirty adult male albino rats of local strain with body weight (b. wt.) ranging between (120-140 gm) were used in the current work . Rats were divided into three equal groups: 38

Group I (Control group), were given subcutaneous (s.c) saline solution (0.01ml /100gm b. wt.).

Group II (Diabetic group), were given s.c alloxan (120 mg / kg b. wt.) in order to induce diabetes mellitus ***(Dunn et al., 1943)***.

Group III (BCB treated group), were given alloxan to induce diabetes then given Bosswellia-water extract (0.01 g/100 gm b. wt.)orally once daily for one month.

B-Drugs and chemicals:

Alloxan (powder from B.D.H chemical LTD, England), dissolved in acetate buffer (pH 5.5) prepared immediately before use.

Trichloroacetic acid (Merck) crystals were used for determination of liver glycogen.

Bosswellia carterii resin, brought from the local market for preparing water extract.

Methods:

- ***Induction of diabetes mellitus:*** By giving s.c freshly prepared alloxan solution 120 mg / kg after an overnight fasting of the animals. After 48 hours blood glucose level was determined by glocometer. Rats with blood glucose level ranging from 180 to 250 were considered diabetic ***(Dunn et al., 1943)***.

- ***Preparation of water extract of Boswellia carterii***: 50 grams of the dry resin of the plant was boiled in 100 ml of distilled water for 10 minutes. After cooling to room temperature it was filtered and stored in a refrigerator till the time of use.

- ***Preparation of serum and determination of various parameters***: The body weight of each rat was determined at the beginning of the experiment. After thirty days of the experiment, body weight of five rats of each group was determined, blood was withdrawn from the retrobulber venous plexus, left to clot then centrifuged to separate serum. The remaining five rats of each group were left for another two weeks without any additional treatment then body weight was determined and serum was prepared as mentioned before. Blood glucose was determined ***(Teitz, 1986),*** and also serum insulin ***(Reeves, 1983).***

At the end of each period, animals were sacrificed, livers were taken for determination of liver glycogen ***(Joseph, 1955***). Pancreases were taken, stained with Hematoxylin and Eosin (HX & E) and modified aldhyde fuchsin ***(Halami, 1952)*** for histological study.

Student (t) test was used to compare between groups, $P < 0.05$ was considered significant ***(Snedecor and Cochron, 1980***).

Results

As shown in table (1), alloxan led to significant decrease in body weight, liver glycogen and serum insulin with significant increase in blood glucose level ($P < 0.01$) as compared to control group. BCB treatment led to significant increase in body weight, liver glycogen and serum insulin with significant decrease in blood glucose level ($P < 0.01$) in BCB treatment group as compared to alloxan-induced diabetic group. On the other hand, no significant changes in these parameters was recorded when compared to control group (except body weight where it was significantly increase ($P < 0.05$) after the treatment period only). In the recovery group there was significant increase in body weight liver glycogen and serum insulin with significant decrease in blood glucose level ($P < 0.01$) as compared to alloxan-induced diabetic group.

Histological examination of slides of pancreas stained with Hx & E of control group showed normal pancreatic islets with rich vascular supply . In the islets, cells were arranged in irregular cords with blood capillaries in between. Modified aldhyde fuchsin stain showed the three main types of cells of the pancreatic islets (alpha, beta and delta cells)**.β** cells were more abundant, occupy the central portion of the islet and contain numerous granules. Alpha and delta cells occupying the periphery of the islet. Delta cells were usually adjacent to alpha cells and were somewhat larger in size.

Alpha cells are granular and polygonal with central spherical nuclei (fig. 1a &b). Alloxan administration led to shrinkage of the normal architecture of the pancreatic islets. The cytoplasm of the cells was vacuolated with pyknotic nuclei, many necrotic cells were seen and many cells showed hydropic degeneration (fig.2 a &b).

BCB treatment showed normal architecture of the pancreatic islets. The cytoplasm be come granulated, the vacuoles of **β** cells disappeared and nuclei be come normal (fig. 3 a & b). The improved histolo-gical picture caused by BCB treatment was seen after the recovery period.

The body weight and levels of blood glucose, serum insulin and liver glycogen in control, diabetic and BCB treatment in male albino rats after treated and recovery periods.

Groups / Test parameters		Treated period			Recovery period		
		Control	Diabetic	BCB treated	Control	Diabetic	BCB treated
Body weight (gram)		139 ± 1.6	112 ± 2.04	148 ± 2.6	137 ± 1.4	105± 1.9	141 ± 2.2
P value	A		P < 0.01	P < 0.01		P < 0.01	P < 0.01
	B			P < 0.01			P < 0.01
Blood glucose(mg /dl)		136.8±0.86	266.4±0.41	137 ± 0.85	137.6±0.68	264.8±.86	135.8±1.46
P value	A		P < 0.01	P < 0.01		P < 0.01	P < 0.01
	B			P < 0.01			P < 0.01
Serum insulin (µ / ml)		41.6 ± 1.51	20.6 ± 1.81	37.6 ±1.51	40 ± 1.84	24 ±1.71	37 ± 1.71
P value	A		P < 0.01	P < 0.01		P < 0.01	P < 0.01
	B			P < 0.01			P < 0.01
Liver glycogen (mg / dl)		173 ± 0.9	152 ± 0.7	170.2 ±0.6	171.5 ±0.7	148.7±0.5	176.01±0.43
P value	A		P < 0.01	P < 0.01		P < 0.01	P < 0.01
	B			P < 0.01			P < 0.01

A- In comparison with control group.
B- In comparison with diabetic group.
- Values given are mean ± SE .

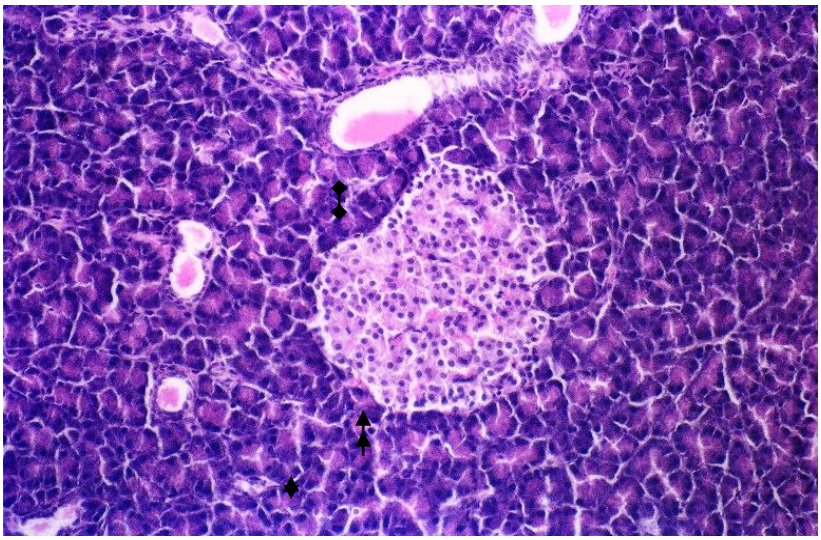

Fig. (1 -a) A photomicrograph of pancreas of control rat stained with HX & E stain shows normal islet cells architecture ▲ pancreatic acini ▲ and blood vessels ↕ (X 400).

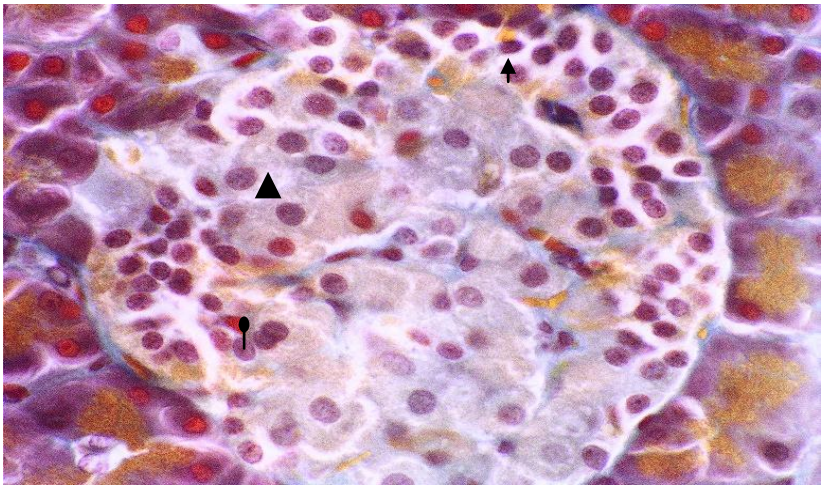

Fig. (1-b) A photomicrograph of pancreas of control rat stained with modified aldhyde fuchsin stain shows normal β cells (▲), delta cell ( ) and alpha cell ( ) of islets of Langerhans (X 1000).

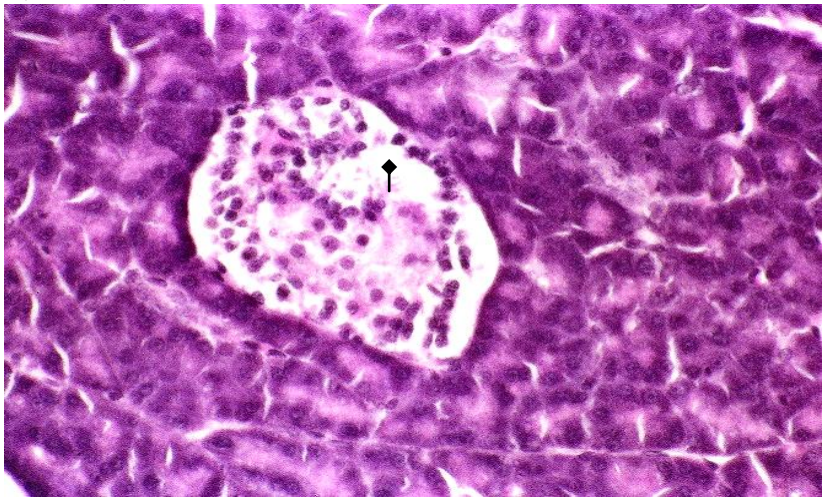

Fig. (2- a)A photomicrograph of pancreas of alloxan-induced diabetic rat stained with HX & E stain shows shrunken islet architecture and necrosis (X 400).

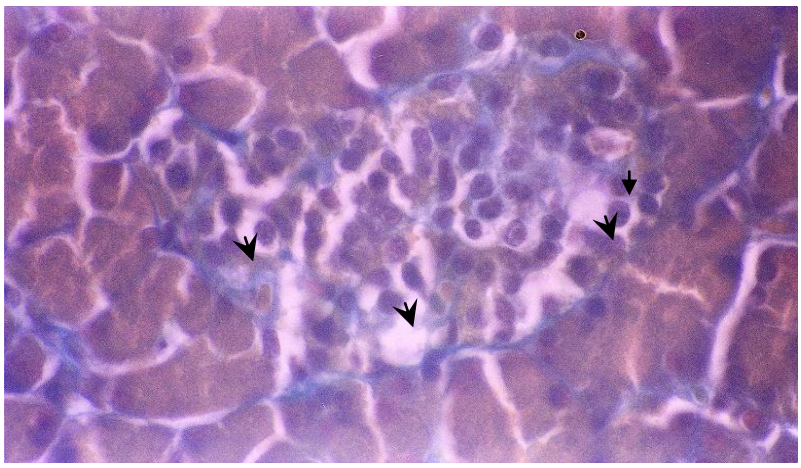

Fig. (2- b) A photomicrograph of pancreas of alloxan-induced diabetic rat stained with modified aldhyde fuchsin stain shows degeneration of β cell ↓ with cytoplasmic vacuoles ↓ (X 1000).

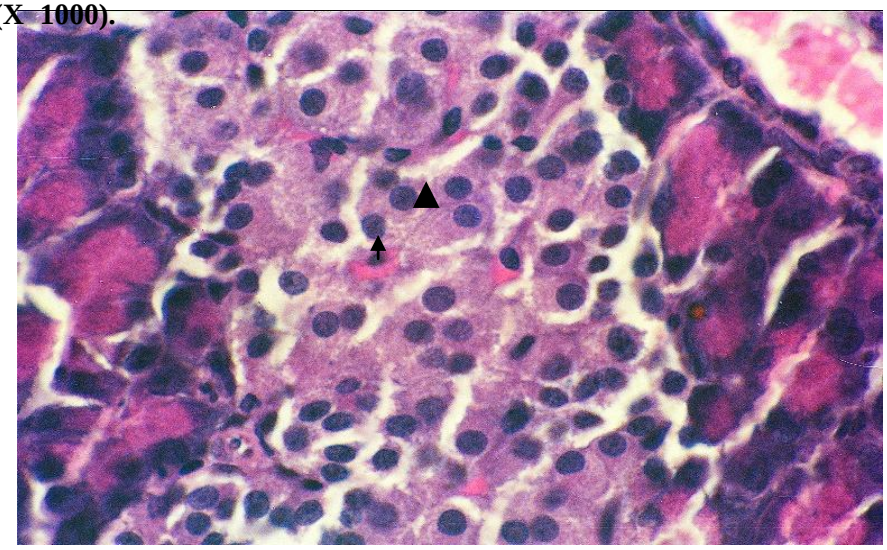

Fig. (3- a) A photomicrograph of pancreas of rat of BCB treated group stained with HX & E shows restoration normal islets architecture β cell ▲ and alpha ↑ cell (X 400).

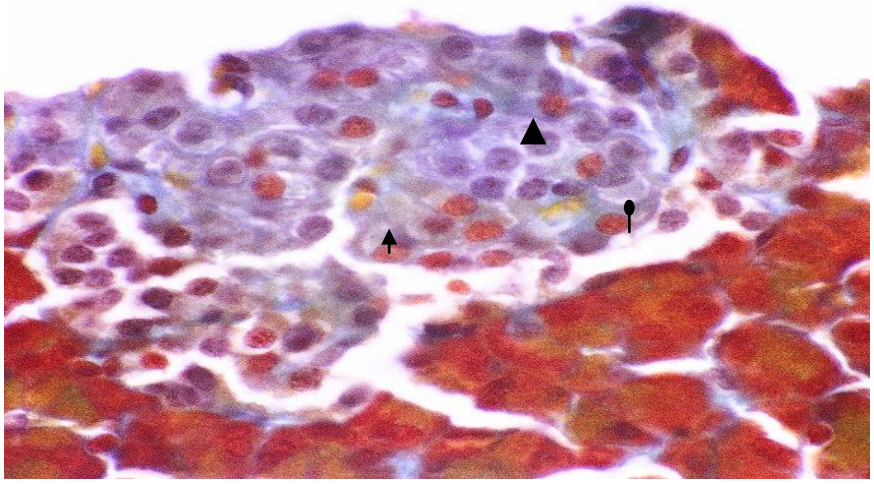

Fig. (3- b) A photomicrograph of pancreas of rat of BCB treated group stained with modified aldhyde fuchsine stain show regeneration of islet β cells ▲ , delta cell and alpha cell ↑ (X 1000).

Discussion

In the present study the alloxan induced-diabetic rats showed decrease in insulin secretion which may be due to the selective toxic effect of alloxan on the beta cells of islets of Langerhans ***(Bolaffi et al., 1986)***. Alloxan has direct inhibitory effect on ionic pump of the cell membrane leading to increase in the cell size. It also inhibit intracellular energy production leading to decreased insulin synthesis and secretion ***(Majno and Joris, 1999)***.

The decrease in the body weight in the diabetic rats may be explained by accelerated conversion of protein into H_2O and CO_2 plus diminished protein synthesis which lead to protein depletion and wasting

(*Gannong, 2003*). Decreased body weight may also be explained by mobilization of fat from its stores with decreased lipogenesis leading to decreased body fat content **(*Nishikawa et al., 2000*).** The decreased liver glycogen in diabetic rats may be due to the increase of glycogenolysis with increased liver glucose output during insulin deficiency ***(Gold ,1970)***. It also may be due to decreased glycogenesis as a result of decreased glycogen synthetase activity and /or increased activity of glucose -6-phosphatase **(*Sheela and Augusti, 1992*)**.

The present study showed diminished in size of islets of Langerhans and greatly damage in **β** cells in diabetic rats. Where alloxan acts as a specific islet **β** cells toxin. This may be deleterious effects of alloxan on permeability, transport, intracellular energy generation and insulin secretion which should be attributed to free radical formation which damage various cellular constituents and cytoplasmic vaculation **(*Malaisse,1982*)**. Vaculation of the cells is the most prominent lesion associated with functional islet abnormality and development of hyperglycemia **(*Bolaffi et al., 1986*)**. And may be also attributed to the ability of alloxan to inhibit enzymes of the tricarboxylic acid cycle and Ca^2 dependent dehydrogenases in **β** cell mitochondria, causing ATP deficiency, cessation of insulin production and cell necrosis **(*Shafri*r, *2003*)**.

Treatment with BCB led to correction of the hypoinsulinaemia which my be due to the regeneration of the **β** cells of islets of Langerhans as shown histologically. BCB treatment showed normal architecture of the **β** cells, cytoplasmic granules with disappearance of the cytoplasmic vaculation in **β** cells. This result may be explained by the metaplastic change of the ductal or acinar epithelial cells of the pancreas to islet cells under unknown stimulus ***(Hisoha and Horie, 1990)***. BCB may have direct stimulatory effect on **β** cell division and / or contain non metabolizable 2-deoxy 3-O-methylglucose which block the diabetogenic effect of alloxan ***(Shafrir, 2003)***. And also BCB may have direct protective effect on **β** cells through its antioxidant action **(*Altman et al., 2004*)**.

The increased insulin secreation caused by BCB may be through stimulation of secretion of the Golgi complex ***(Bever and Zahand, 1979)*** or it may be possibly through adirect effect on intracellular calcium transport ***(Campbell et al., 1991)***.

Correction of body weight loss may be due to increased usage of glucose as a source of energy instead of fats and proteins secondary to increased serum insulin ***(Nishikawa et al., 2000)***. Body weigh gain may also be explained by the effect of BCB on the gastrointestinal tract as the plant increases gastrointestinal motility and secretion ***(Chevallier, 1996*** and ***Duke, 2002)***. The shift of metabolic pathway towards carbohydrate as a source of energy with saving proteins and fats as well as their increased biosynthesis led to increased body weight **(*Peavy et al., 1985*).** The increased liver glycogen in B.C.B treated rats may be due increased insulin with subsequent increase of glycogen synthetase activity as well as hepatic hexokinase and glycogen-6-phosphatase activity ***(Sheela and Augusti, 1992).***

From the present study, it is clear that other investigations on plant toxicity and usage of different dosage and periods as well as its effect on vital organs must be carried out before recommendation of its usage in treatment of diabetes mellitus.

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تأثير مستخلص اللبان على أيض الكربوهيدرات في ذكور الجرذان البيضاء المصابة بالسكر التجريبي .

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فى هذا البحث أستخدم ثلاثون جرذا ابيض لدراسة تأثير اللبان على أيض الكربوهيدرات فى داء البول السكرى قسمت إلي ثلاث مجموعات متساوية – المجموعة الأولى ضابطة ، والمجموعة الثانية تم حقنها بالألوكسان لأحداث داء السكري ، والمجموعة الثالثة تم معالجتها باللبان لمدة شهر بعد أحداث داء السكري بها . وقد تم قياس نسبة السكر والأنسولين فى الدم والجليكوجين فى الكبد ووزن الجسم لكل المجموعات كذلك تم عمل فحص مجهرى لشرائح البنكرياس . كما تضمنت الدراسة ايضا تقييم نفس القياسات بعد فترة الأستشفاء (خمسة عشرة يوما بدون علاج إضافي) .

وقد أ دى الحقن بعقار الألوكسان الى تدمير خلايا بيتا مما ادى الى نقص ذو دلالة إحصائية في هرمون الأنسولين و جليكوجين الكبد وكذلك وزن الجسم مع زيادة ذات دلالة إحصائية في نسبة السكر فى الدم .

وقد أ دت المعالجة باللبان إلى تجديد خلايا بيتا مع زيادة ذات دلالة إحصائية في هرمون الأنسولين وجليكوجين الكبد وتحسن فى وزن الجرذان مع انخفاض ذو دلالة إحصائية في نسبة السكر فى الدم وقد امتدت هذه النتائج الإيجابية حتى بعد توقف العلاج – أى إلى فترة الاستشفاء مقارنة بالمجموعة المصابة بمرض السكر .

ويستخلص من هذا البحث أن لنبات اللبان تاثير جيد على خلايا بيتا وافراز الأنسولين وتحسن وزن الجسم وجليكوجين الكبد وخفض نسبة السكر فى الدم ونوصى بمزيد من الأبحاث بجرعات مختلفة من النبات وكذلك دراسة الأثار الجانبية للنبات - إن وجدت –على اعضاء الجسم المختلفة قبل الوصول إلى رأى قاطع حول استخدام هذا النبات لعلاج مرض السكر.