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Original Research Article

Antidiabetic and antioxidant properties of *Boswellia sacra* oleo-gum in streptozotocin-induced diabetic rats

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ABSTRACT

Background: Diabetes is a metabolic disorder requiring the administration of insulin or other oral hypoglycemic medicines. Although metformin is a popular prescription for type 2 diabetes, long-term use of chemotherapy-based diabetes medications can be hazardous. As a result, novel plant medicines with a high concentration of bioactive molecules, no harmful side effects, and potent pharmacological effects must be found. Edible Boswellia sacra (*B. sacra*) Flueck oleo-gum resin is widely utilized to treat many clinical diseases in traditional Arab, Chinese, African, and Ayurvedic medicine.

Objective: The goal of this study was to examine the possible therapeutic benefits of several *B. sacra* oleo-gum resin extracts on rat streptozotocin (STZ)-induced hyperglycemia (Type II).

Materials and methods: For 29 days, hyperglycemic rats are given either metformin (the reference drug; 250 mg/kg body weight per day) or several *B. sacra* extracts (ethanol, methanol, hydrodistilled, ethyl acetate, and acetone extracts) at doses of 200 or 400 mg/kg/day. Blood glucose levels and body weights were measured before the initiation and at 7, 11, 16, 22, and 29 days after oral treatment. Furthermore, an oral glucose tolerance test (OGTT) was carried out. At the end of the study, the rats were euthanized, and blood samples were obtained to evaluate cytokines (interleukin (IL-)2 and IL-8), reduced glutathione (GSH), superoxide dismutase (SOD), and serum insulin levels. The pancreas and liver tissues were rapidly excised, washed, fixed, and kept in a 10% formalin buffer for histological examination.

Results: *B.* sacra's ethanolic extract had the greatest concentration of total pentacyclic triterpenic acid (PTA) (391.52 mg/g) in comparison to the other extracts. The lower dose of *B. sacra* ethanol extract, 200 mg/kg/day, reduces blood glucose levels more efficiently than the higher dose of 400 mg/kg/day. In a 180-min OGTT, diabetic rats given ethanol extract (200 mg/kg) performed no better than control rats and even outperformed those given the reference medication metformin. Additionally, ethanol extract (200 mg/kg)- or metformin-treated diabetic rats gained weight. This was associated with a significant ($p < 0.05$) decrease in serum levels of IL-2 and IL-8, a reduction in oxidative stress as evidenced by a significant ($p < 0.05$) increase in SOD and GSH compared to the untreated diabetic group, and a significant ($p < 0.05$) increase in serum insulin levels compared to normal plasma rat levels. These discoveries, which were eventually confirmed by histochemical assays, indicated that the ethanol extract of *B. sacra* greatly enhanced the cellular architecture of pancreatic and liver cells.

Conclusion: The present investigation indicates that the ethanol extract of *B. sacra* oleo-gum resin, which contains a high proportion of acetyl-β-boswellic acid (β-ABA) and acetyl-11-keto-β-boswellic acid (AKBA), possesses antihyperglycemic, anti-inflammatory, and anti-oxidant properties for the first time to our knowledge. Additionally, it restores hepatic cells in STZ-induced diabetic rats and protects the pancreas against oxidative damage. Thus, the current study's results give a scientific rationale for the use of *B. sacra* in the medical management of

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diabetes and associated complications. More investigation into the metabolic profiles of these extracts must be conducted to establish the precise mechanism of action of the ethanol extract.

1. Introduction

Diabetes is a long-term metabolic disease that necessitates the use of oral hypoglycemic medications such as insulin. Diabetes causes death and disability over time by harming, malfunctioning, and failing many organ systems, such as the heart, blood vessels, eyes, kidneys, and nerves [1]. In 2016, diabetes was the seventh largest cause of mortality, accounting for an estimated 1.6 million fatalities from the disease itself and an additional 2.2 million deaths from high blood sugar [2], with that number expected to quadruple by 2030. Furthermore, according to the WHO, 366 million people worldwide will be affected by this disorder by 2030 [3].

Despite substantial advances in improving the prognoses of diabetic patients, a cure is still a pipe dream. Metformin is a common medication for type 2 diabetics with high blood sugar levels. When chemo-based drugs are administered to treat diabetes for a long time, toxicity develops. As a result, new substances must be recognized as plant medicines with a high concentration of bioactive compounds that have significant pharmacological effects without any negative side effects [4].

Plant-based preparations are the mainstay of all available medicines, especially for diabetic poor people, because of their ease of availability and low cost. Furthermore, plant-based traditional health therapies are used by approximately 80–90% of the world's population due to their accessibility and therapeutic safety [5].

Chemokines control leukocyte migration to locations of tissue injury, which aids in inflammatory processes [6]. Inflammatory cytokines released by invading immune cells, such as interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α), have been demonstrated to contribute to pancreatic cell death and consequently diabetes induction [7]. Furthermore, reactive oxygen species (ROS) trigger cell apoptosis, impede insulin production, and increase insulin resistance in type 2 diabetes [8].

Researchers from all over the world have been searching for medical plants as a source candidate globally for decades, and their application in drug development has grown to promote the treatment of diabetes because these plants contain phytoconstituents such as flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides that may have anti-diabetic properties [9]. In traditional Arab, African, Ayurvedic, and Traditional Chinese medicine, *Boswellia* of the Burseraceae family is frequently used to treat a variety of disorders including diabetes, rheumatoid arthritis, asthma, and Crohn's disease [10].

The most common species responsible for commercially available frankincense (olibanum) are *B. sacra, B. carteri, B. frereana, B. papyrifera, B. dalzielii hutch,* and *B. serrata*.

Administration of extracts derived from various *Boswellia* species, including *B. serrata* [11,12], *B. dalzielii* Hutch [13], and *B. carteri* Birdw [14], dramatically lowered blood glucose levels in diabetic rats. This could be attributed to an animal model inhibiting cytokine production or action (IL-1A, IL-1B, IL-2, IL-6, IFN-γ, TNF-α, and oxidative stress) in an animal model, which prevents islet destruction and subsequent hyperglycemia [15].

It is worth noting that studies on Boswellia species are currently underway, and further investigation is required to ascertain the safety and effectiveness of using these plants to treat diabetes. The current study evaluated the antihyperglycemic effects of Omani frankincense *B. sacra* extracts to metformin, which served as the control treatment in STZ-induced type 2 diabetic rats.

2. Materials and methods

2.1. Drugs and chemicals

Metformin HCl (98+%), the reference medication, bought from Thermo Fisher Scientific in Germany. Sigma-Aldrich Chemical Co., Poole, UK, supplied the streptozotocin. The remaining chemicals and solvents were of analytical grade.

2.2. B. sacra source

Oleo gum resin was extracted from authenticated *B. sacra* Flueck trees in the Sadah-Dhofar region of Oman, approximately 135 km from Salalah, between November and December 2019–2020. This gum resin (a Najdi sample) was collected using traditional methods and authenticated with a sample preserved in the Nizwa University Herbarium Center in Oman under voucher number LJC29. It should be noted that Boswellia sacra Flueck is a homotypic synonym of Boswellia carteri Birdw.

2.3. Extraction of B. sacra gum resin

The oleo-gum resin was ground into a fine powder with an electrical machine grinder before extraction. Five equal aliquots of plant material were independently extracted using different solvents. Among these are extracts in 100% ethanol, 100% methanol, hydrodistillation (taking acid fraction), acetone, and 100% ethyl acetate. The extracts were stirred in the solvents for 2 h at room temperature before being vacuum-filtered through Whatman's filter paper No. 1 and dried in a vacuum oven at 35 °C until no further mass change was observed.

2.4. Pentacyclic triterpenic acid analysis in B. sacra extracts

Eight different pentacyclic triterpenic acids (PTAs) were used to chemically characterize *B. sacra* extracts. These are α-boswellic acid (α-BA), acetyl-α-boswellic acid (α-ABA), β-boswellic acid (β-BA), acetyl-β boswellic acid (β-ABA), 11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA), lupeolic acid (LA), and acetyl-lupeolic acid (ALA), which were determined using HPLC-MS/MS according to the method of Schmiech et al. [16]. The HPLC-MS/MS method's linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) were all validated (Table 1 and Fig. 1).

Table 1
HPLC-MS/MS validation data: calibration curves (with internal standard), Retention time (T_r), mass-to-charge ratio *m/z*), the retardation factor (R), limit of detection (LOD) and limit of quantification (LOQ).

Substances	T_r [min]	*m/z*	R	LOD [μg/ml]	LOQ [μg/ml]
11-Keto-β-boswellic acid (KBA)	5.4	469.4	0.9997	0.2207	0.8336
Acetyl-11-keto-β-boswellic acid (AKBA)	7.6	511.4	1.0000	0.0722	0.2749
α-Boswellic acid (α-BA)	12.1	455.4	0.9999	0.1518	0.5756
β-Boswellic acid (β-BA)	12.8	455.4	0.9990	0.4071	1.5257
Acetyl-α-boswellic acid (α-ABA)	15.3	497.4	0.9999	0.1573	0.5963
Acetyl-β-boswellic acid (β-ABA)	16.0	497.4	0.9998	0.1730	0.6554
Lupeolic acid (LA)	11.0	–	–	0.0195	0.017
Acetyl-lupeolic acid (ALA)	15.17	–	–	0.00161	0.0060

2.5. Animals

Sprague-Dawley rats aged eight to ten weeks and weighing 180 ± 30 g were obtained from the University of Petra Pharmaceutical Center (UPPC) in Amman, Jordan. Rats were housed in cages in an air-controlled room with a light/dark cycle, constant humidity, and a constant temperature (20 ± 5 °C). A commercial pellet diet and tap water were freely available. The animals were handled per our university's policies throughout the investigation. The Ethics Committee at the University of Petra (UOPEC) reviewed and approved the study's procedure (A2412/2019). The study complied with the ethical standards of the Helsinki Declaration.

2.6. Induction of experimental diabetes

After 7 days of acclimatization and an overnight fast, male rats (n = 60) recieved a single intraperitoneal (i.p.) injection of freshly made streptozotocin (55 mg/kg; STZ) mixed in sodium citrate buffer (0.01 M, pH 4.5 adjusted with HCl 1 N). To avoid hypoglycemia, the rats were given a 10% w/v sucrose solution in their drinking water for the first 24 h, while healthy control animals were given an identical volume of the buffer solution (n = 5).

Blood glucose levels were monitored using venous tail blood and a portable glucometer one week following the injection (to allow for the onset of hyperglycemia). Blood glucose levels more than 11 mmol/L or above 200 mg/dl were used to confirm hyperglycemia (type II) in rats and were considered for the treatment protocol [17].

2.7. Experimental protocol

The diabetic and control rats were divided into 13 groups (n = 5 rats/group). For three weeks, rats were given vehicle (saline solution) or extracts of *B. sacra* oleo gum resin orally through gastric gavage at doses of 200 or 400 mg/kg [11] as follows:

Group I: Healthy, untreated rats served as a negative control, receiving a saline solution (0.1ml/100 gm).
Group II: Non-treated diabetic rats
Group III: Diabetic rats +200 mg/kg ethanol extract
Group IV: Diabetic rats +400 mg/kg ethanol extract
Group V: Diabetic rats +200 mg/kg methanol extract
Group VI: Diabetic rats +400 mg/kg methanol extract
Group VII: Diabetic rats +200 mg/kg hydrodistillation (acid fraction) extract
Group VIII: Diabetic rats + 400 mg/kg hydrodistillation (acid fraction) extract
Group IX: Diabetic rats + 200 mg/kg ethyl acetate extract
Group X: Diabetic rats + 400 mg/kg ethyl acetate extract
Group XI: Diabetic rats + 200 mg/kg acetone extract
Group XII: Diabetic rats + 400 mg/kg acetone extract
Group XIII: Diabetic rats + Metformin, a standard hypoglycemic agent (reference drug; 250 mg/kg body weight per day).

The dosages of 200 and 400 mg/kg indicate 1 and 20% of the lethal concentration (LC50), respectively. All dosages were emulsified with 10% DMSO.

2.8. Oral glucose tolerance test (OGTT)

After an overnight fast, the rats' blood glucose levels were measured. According to Chaimum-aom et al. [18], rats were given glucose solutions (2 g/kg) 1 h after being fed extracts and/or medications. Blood (0.2–0.3 ml) was collected from each rat's tail vein following glucose loading for 0, 30, 60, 120, and 180 min. A portable glucometer was used to measure plasma glucose levels.

2.9. Measurements of body weight and blood glucose

Rat body weights and blood sugar levels were measured at 10:00 a.m. from the tails of fasted rats before the initiation and at 7, 11, 16, 22, and 29 days after oral treatment.

2.10. Blood serum collection and processing of tissues

On day 29, at the end of the study, the rats were anesthetized by inhalation of Floran (isoflurane; Anaesthetic Kent Scientific Corporation, USA). Blood samples were taken directly from the heart and placed in non-heparinized tubes after the chest was opened. Thirty minutes later, blood samples were centrifuged at 1000×*g* for 15 min, and serum was kept at 20 °C to determine cytokines (IL-2 and IL-8), reduced glutathione (GSH), superoxide dismutase (SOD), and serum insulin. Cytokines, GSH, SOD, and serum insulin levels were measured using enzyme-linked immunosorbent assay kits in accordance with the manufacturer's instructions (ELIS; ThermoFisher, USA and Europe).

All serum samples were examined in duplicate for each test in a single assay. The IL-2 sensitivity was 11 pg/ml, with intra- and inter-assay being <5 % and <10% respectively; the IL-8 sensitivity was 1.0 pg/ml, intra- and inter-assay were <6 % and <8% respectively; GSH sensitivity was <0.5 μg/ml, with intra- and inter-assay being ≤8 % and ≤12% respectively; the SOD sensitivity was 1U/ml, intra- and inter-assay being ≤8 % and ≤12% respectively; and the insulin sensitivity was 46.87 pg/ml, with intra- and inter-assay being ≤8 % and ≤10% respectively. For histological examination, the pancreas and liver were rapidly excised, rinsed in icy-cold phosphate buffer solution (PBS), fixed, and kept in 10% formalin buffered with PBS pH 7.4.

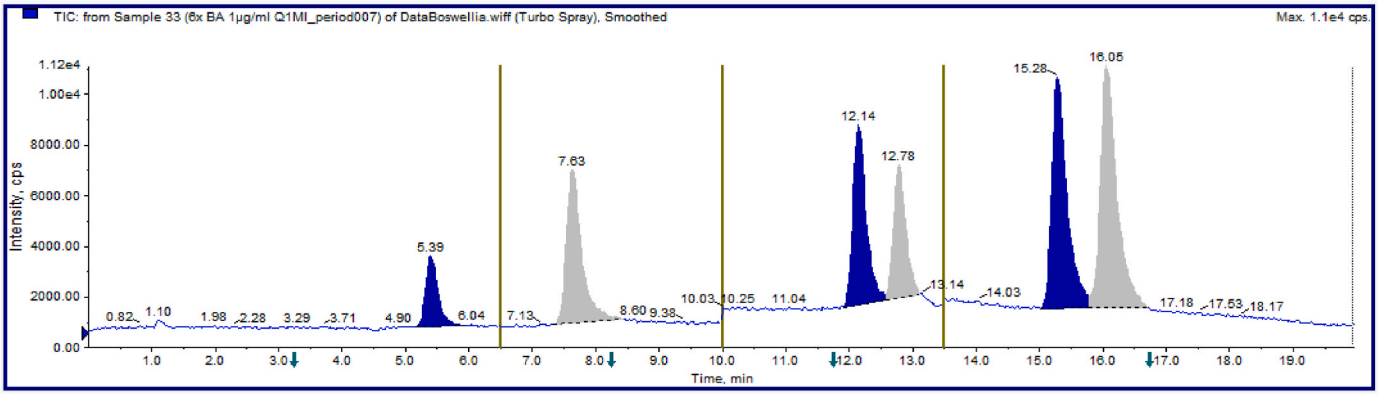

Fig. 1. HPLC-DAD-MS/MS chromatograms of Pentacyclic Triterpenic Acids (PTAs) extracted from *B. sacra*.

2.11. Histological study

The pancreas and liver tissues were dehydrated in descending isopropanol grades before being cleared in xylene. The tissues were then encased in molten paraffin wax. Next, a series of transverse sections (5 μm) were cut and stained with hematoxylin and eosin. The specimens were examined under a 400-fold magnification light microscope.

2.12. Statistical analysis

The current study's data were statistically analyzed by a biostatistician using SPSS statistical software version 26 (SPSS Inc., Chicago, IL, USA). All data were tested for normal distribution by the Anderson-Darling test and equality of variances by Levene's test. One-way ANOVA was used for statistical comparison, followed by Dunnett's multiple comparison Test. Data were presented as means ± standard error of means (SEM). When $p < 0.05$, the values were considered statistically significant.

3. Results

3.1. Analysis of pentacyclic triterpenic acids in B. sacra extracts

The findings of the HPLC-MS/MS analysis of total PTA content using an ethanolic extract of *B. sacra* oleo gum resin revealed the greatest concentration (391.52 μg/mg) compared to the other extracts, followed by methanol extract (323.36 μg/mg), while acetone extract exhibited the lowest total PTA (237.02 μg/mg) (Table 2). We observed that acetyl-β boswellic acid (β-ABA) was the most abundant in all extracts used for *B. sacra* oleo gum resin, followed by acetyl-11-keto-β-boswellic acid (AKBA).

3.2. Effect of different extracts of B. sacra on OGTT

The blood glucose levels in control, diabetic, diabetic-treated with various *B. sacra* extracts, and diabetic metformin-treated rats following oral administration of glucose (2 g/kg) are shown in Table 3.

Blood glucose levels in all groups were significantly lower at zero time, just before oral administration of glucose, than in the diabetic group. There were no significant changes after oral glucose delivery between diabetic rats given methanol extract (400 mg/kg) and diabetic rats that did not receive any therapy. All groups reached their maximal blood glucose levels 30 min after receiving glucose. Even though blood glucose levels remained elevated after 2 h, they began to fall after that. Blood glucose levels were significantly lower in all groups than in the diabetic group 180 min after oral glucose administration. After 30 min, an ethanol extract of *B. sacra* at 200 mg/kg (Group 3) significantly improved glucose tolerance, and the level of glucose in their blood matched that of the control and diabetic metformin-treated rats (Group 13). All diabetic rats treated with various *B. sacra* extracts and diabetic rats given metformin had significantly higher blood glucose levels than the control group and significantly lower levels than the diabetic group at various time points, with the exception of the ethanol extract at 200 mg/ml at 180 min. When diabetic rats were administered 200 mg/kg of ethanol extract 180 min after the OGTT, they did not differ significantly from the control group and even outperformed rats given the reference medicine metformin.

3.3. Body weight changes in diabetic rats following oral administration of different B. sacra extracts

Before the commencement of the experiment, the body weights of all groups were comparable (Table 4).

After 7 days, diabetic rats receiving no treatment, diabetic rats with treatment, and diabetic rats with metformin had significantly lower body weights ($p < 0.05$) compared to control rats.

The difference in final weight between diabetic-treated and diabetic-untreated rats is statistically significant ($p < 0.05$) when compared to control or diabetic rats treated with ethanol extract (200 mg/kg) or metformin. (p 0.05).

Furthermore, there were no significant changes in body weight in diabetic rats given 200 or 400 mg/kg of *B. sacra* extract.

Diabetes rats given ethanol extract (200 mg/kg) or metformin both gained weight as determined by the percentage of weight loss over time relative to their initial weight; however, rats given ethanol and methanol extracts (200 mg/ml) lost less weight, with ethanol extracts being more predominant (13.1% vs. 17.2%, respectively) (Table 4). During the experiment, we observed no signs of diarrhea, weakened condition, or exhaustion in either the control or all treated group rats.

3.4. The changes in fasting blood glucose levels over the time course

Blood glucose levels in the control, diabetic not treated, and diabetic-treated groups (either with *B. sacra* extracts or metformin) were not comparable before treatment commenced (at zero time). The blood glucose levels of diabetic rats that were not treated, those who received different *B. sacra* extracts, those who received metformin, and the control group are shown in Table 5.

At day 7, blood glucose levels in all diabetic groups receiving *B. sacra* extracts were significantly ($p < 0.05$) higher than in those receiving metformin. However, on days 11, 16, 22, and 29, diabetic groups treated with *B. sacra* extracts had either significantly ($p < 0.05$) lower or non-significant blood glucose concentrations than those treated with metformin. Diabetic rats that were not treated had significantly ($p < 0.05$) higher blood glucose levels compared to control or diabetic-treated rats (either *B. sacra* extracts or metformin).

On days 11, 16, 22, and 29, oral administration of ethanol extract (200 mg/kg) significantly ($p < 0.05$) decreased blood glucose levels. After four weeks of treatment, on day 29, all diabetic rats that received a 200 mg/kg/day ethanol extract of *B. sacra* had significantly ($p < 0.05$) lower blood glucose levels compared to diabetic non-treated groups, diabetic-treated groups with other extracts, or diabetic-treated rats with metformin, with blood glucose concentrations comparable to control rats. The lower dose of *B. sacra* ethanol extract, 200 mg/kg/day, is more

Table 2
Analysis of pentacyclic triterpenic acids (PTA) in *B. sacra* extracts

Sample	Conc. (μg/mg) (w/w)								
	α –BA	α -ABA	β -AB	β -ABA	KBA	AKBA	LA	ALA	Total
Ethanol Extract	19.850	65.400	50.600	111.000	2.560	73.200	9.010	59.900	391.52
Methanol Extract	18.500	52.800	43.850	92.250	2.555	58.500	8.305	46.600	323.36
Acid Fraction	14.850	49.900	40.250	86.200	3.320	50.400	8.660	42.550	296.13
Ethyl acetate Extract	14.700	38.700	36.050	67.050	3.870	56.000	6.040	27.150	249.56
Acetone Extract	11.615	36.050	30.900	63.350	4.055	54.550	5.645	30.850	237.02

Table 2. Concentrations of pentacyclic triterpenic acids (PTA) in resins of *Boswellia* oleo-gum from different extracts: α-boswellic acid (α-BA), acetyl-α-boswellic acid (α-ABA), β-boswellic acid (β-BA), acetyl-β boswellic acid (β-ABA), 11-keto- β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA), lupeolic acid (LA), and acetyl-lupeolic acid (ALA).

Table 3
Effect of different extracts of *B. sacra* on OGTT

Group	Zero min	30 min	60 min	90 min	120 min	180 min
C	96.4 ± 4.05[a]	125 ± 1.6[a]	120.5 ± 38.14[a]	117.2 ± 6.35[a]	113.8 ± 9.98[a]	109.2 ± 3.9[a]
DNT	509.6 ± 19.8	590.6 ± 8.48	589.8 ± 6.71	553.6 ± 27.64	531.8 ± 35.78	452.6 ± 25.7
D + EE (200 mg/kg)	105.6 ± 8.02[a]	269.8 ± 19.37*[a]	251.4 ± 10.77[a]	214 ± 12.07*[a]	194 ± 34.1*[a]	114.6 ± 4.05[a]
D + EE (400 mg/kg)	164.4 ± 58.58[a]	323.2 ± 32.64*[a]	314.6 ± 33.51*[a]	295.4 ± 35.68*[a]	269.4 ± 29.7*[a]	215.2 ± 20.43*[a]
D + ME (200 mg/kg)	101.4 ± 2.73[a]	356 ± 17.27*[a]	336.4 ± 35.17*[a]	331 ± 28.51*[a]	320.4 ± 29.73*[ab]	293.6 ± 18.9*[ab]
D + ME (400 mg/kg)	130 ± 35.68[a]	545.8 ± 35.6*	533.8 ± 31.57*[b]	513.6 ± 49.42*[b]	437.4 ± 71.83[ab]	395 ± 18.25*[ab]
D + AFE (200 mg/kg)	106.2 ± 16.51[a]	315.4 ± 34.87*[a]	300.6 ± 30.01*[a]	295.4 ± 17.37*[a]	284.4 ± 98.8*[a]	263 ± 58.57*[a b]
D + AFE (400 mg/kg)	137.6 ± 31.38[a]	332.2 ± 72.64*[ab]	321.2 ± 33.65*[a]	314.6 ± 33.4*[a]	307.6 ± 34.08*[a]	302.4 ± 30.37*[ab]
D + EAE (200 mg/kg)	152 ± 46.49[a]	354.8 ± 49.30*[a]	348.8 ± 41.37*[a]	344 ± 47.44*	280.2 ± 48.48*[ab]	275.4 ± 62.95*[ab]
D + EAE (400 mg/kg)	123.4 ± 32.36[a]	440.8 ± 25.14*[a]	426 ± 44.90*[b]	413 ± 44.34*	403 ± 29.28*[ab]	371 ± 4.42*[ab]
D + AE (200 mg/kg)	105.8 ± 16.75[a]	314 ± 74.22*[ab]	300.8 ± 42.03*[a]	271.6 ± 53.27[a]	263.2 ± 28.39*[a]	199 ± 25.11*[a b]
D + AE (400 mg/kg)	104.8 ± 9.18[a]	313.2 ± 24.22*[ab]	302 ± 24.58*[a]	301.4 ± 16.64*[ab]	299.6 ± 11.31*[a]	279.2 ± 19.45*[ab]
D + M	122.8 ± 17.88[a]	374.8 ± 40.7[a]	294.8 ± 38.38*[a]	182.8 ± 41.24[a]	133 ± 14.74[a]	133 ± 19.42[a]

Results represent mean ± SEM.
Group C, DNT, D + EE (200 mg/kg), D + EE(400 mg/kg), D + ME (200 mg/kg), D + ME (400 mg/kg), D + AFE (200 mg/kg), D + AFE (400 mg/kg), D + EAE (200 mg/kg), D + EAE (400 mg/kg), D + AE (200 mg/kg), D + AE (400 mg/kg), D + M represent Control, Diabetic non-treated, Diabetic + ethanol extract (200 mg/kg), Diabetic + ethanol extract (400 mg/kg), Diabetic + methanol extract (200 mg/kg), Diabetic + methanol extract (400 mg/kg), Diabetic + acid fraction extract (200 mg/kg), Diabetic + acid fraction extract(400 mg/kg), Diabetic + ethyl acetate extract (200 mg/kg), Diabetic + ethyl acetate extract (400 mg/kg), Diabetic + acetone extract (200 mg/kg), Diabetic + acetone extract (400 mg/kg), and Diabetic + Metformin (reference drug) treated diabetic rats respectively.
< 0.05 indicates a significant difference when compared to the control group.
a< 0.05 indicates a significant difference when compared to the diabetic non-treated group.
b < 0.05 indicates a significant difference when compared to the diabetic treated with Metformin (reference drug) group.

Table 4
Body weight changes in diabetic rats following oral administration of different *B. sacra* extracts

Group	Initial B. Wt. at 0 day	B. Wt. at 7 days	Final B. Wt at 29 days	% of B. Wt reduction in diabetic rats
C	267.6 ± 18.5	285.4 ± 11.0	315.8 ± 15.8	
DNT	247.4 ± 15.7	175.6 ± 10.5*	178.3 ± 8.9*[a]	27.9
D + EE (200 mg/kg)	247.0 ± 10.8	192.2 ± 10.3*	214.6 ± 8.6*	13.1
D + EE (400 mg/kg)	240.8 ± 8.9	185.0 ± 6.3*	185.2 ± 6.9*[a]	23.1
D + ME (200 mg/kg)	232.4 ± 2.8	183.2 ± 7.2*	192.4 ± 5.3*[a]	17.2
D + ME (400 mg/kg)	227.2 ± 9.8	177.8 ± 10.7*	178.2 ± 9.8*[a]	21.5
D + AFE (200 mg/kg)	225.8 ± 7.3	179.4 ± 9.8*	184.4 ± 6.8*[a]	18.3
D + AFE (400 mg/kg)	224.8 ± 5.0	172.0 ± 6.5*	173.2 ± 6.8*[a]	22.9
D + EAE (200 mg/kg)	257.0 ± 18.7	192.6 ± 12.9*	182.2 ± 8.8*[a]	29.2
D + EAE (400 mg/kg)	231.2 ± 8.4	173.6 ± 6.1*	188.3 ± 3.2*[a]	18.6
D + AE (200 mg/kg)	238.0 ± 8.9	172.2 ± 8.9*	174.4 ± 7.8*[a]	29.9
D + AE (400 mg/kg)	228.0 ± 25.2	185.0 ± 18.4*	182.4 ± 8.0*[a]	20.0
D + M	246.25 ± 11.2	193.0 ± 10.5*	210.8 ± 5.5*	14.4

Results represent mean of 5 rats ± SEM.
Group C, DNT, D + EE (200 mg/kg), D + EE(400 mg/kg), D + ME (200 mg/kg), D + ME (400 mg/kg), D + AFE (200 mg/kg), D + AFE (400 mg/kg), D + EAE (200 mg/kg), D + EAE (400 mg/kg), D + AE (200 mg/kg), D + AE (400 mg/kg), D + M represent Control, Diabetic non-treated, Diabetic + ethanol extract (200 mg/kg), Diabetic + ethanol extract (400 mg/kg), Diabetic + methanol extract (200 mg/kg), Diabetic + methanol extract (400 mg/kg), Diabetic + acid fraction extract (200 mg/kg), Diabetic + acid fraction extract(400 mg/kg), Diabetic + ethyl acetate extract (200 mg/kg), Diabetic + ethyl acetate extract (400 mg/kg), Diabetic + acetone extract (200 mg/kg), Diabetic + acetone extract (400 mg/kg), and Diabetic + Metformin (reference drug) treated diabetic rats respectively.
< 0.05 indicates a significant difference when compared to the control.
a< 0.05 indicates a significant difference when compared to the control, and diabetic rats treated with either ethanol extract (200 mg/kg) or metformin.

effective in decreasing blood glucose levels than the higher dose of 400 mg/kg/day.

3.5. Changes in cytokines, antioxidants, and insulin secretion after oral administration of different B. sacra extracts on diabetic rats

According to the findings of this study, the untreated diabetic group had significantly ($p < 0.05$) higher serum levels of IL-2 and IL-8 compared to the control group. Oral treatment with several *B. sacra* extracts at doses of 200 and 400 mg/kg or metformin resulted in a significant ($p < 0.05$) drop in serum IL-2 and IL-8 as compared to the non-treated diabetic group. At 200 mg/ml, ethanol, and ethyl acetate extracts were more effective in normalizing IL-2 and IL-8 serum levels, respectively.

Table 6 shows the effects of various *B. sacra* extracts on SOD enzyme activity and GSH in diabetic rats. GSH and SOD levels in diabetic non-treated rats were significantly ($p < 0.05$) lower than in control rats or

Table 5
Fast blood glucose (FBG) at different time points following

Mean ± SEM	Zero time FBG	7 Day FBG	11 Day FBG	16 Day FBG	22 Day FBG	29 Day FBG
C	83.8 ± 5.56	82.4 ± 1.21[ab]	84.2 ± 3.28 [ab]	83.8 ± 7.71[ab]	86.6 ± 3.98[ab]	80.6 ± 6.02[ab]
DNT	88 ± 2.7	407.8 ± 5.62*[b]	409 ± 11.9*[b]	369.2 ± 19.77*[b]	509.8 ± 19.94*[b]	467.6 ± 39.98*[b]
D + EE (200 mg/kg)	93.2 ± 2.43	380.2 ± 8.78*[ab]	91.6 ± 4.76[ab]	74.4 ± 5[ab]	97 ± 6.07[a]	92.2 ± 5.17[ab]
D + EE (400 mg/kg)	84.2 ± 5.11	426.6 ± 21.57*[ab]	90.2 ± 5.74[ab]	108.6 ± 8.45[ab]	118.4 ± 15.63[a]	209 ± 46.42*[a]
D + ME (200 mg/kg)	88.4 ± 5.83	308.6 ± 30.1*[ab]	102.8 ± 16.19[ab]	99.2 ± 8.56[ab]	107.6 ± 22.55[a]	150.2 ± 41.83*[a]
D + ME (400 mg/kg)	87.4 ± 4.32	424.4 ± 29.81*[b]	194.8 ± 24.79*[a]	130 ± 5.31*[ab]	215.2 ± 7.60*[ab]	211 ± 39.72*[a]
D + AFE (200 mg/kg)	75.2 ± 9.12	366.4 ± 9.8*[ab]	78 ± 6.18[ab]	107 ± 13.24[ab]	106.2 ± 16.52[ab]	123.8 ± 19.71*[a]
D + AFE (400 mg/kg)	85.2 ± 5.12	414.2 ± 22.66*[b]	141.4 ± 4.17*[a]	201.2 ± 28.88*[a]	147.6 ± 21.39*[a]	163 ± 41.09*[a]
D + EAE (200 mg/kg)	86.2 ± 6.9	393.8 ± 6.83*[ab]	172.4 ± 13.94*[a]	211.4 ± 9.56*[a]	195 ± 36.4*[a]	171.4 ± 38.65*[a]
D + EAE (400 mg/kg)	84 ± 10.16	422.4 ± 35.11*[ab]	198.8 ± 25.05*[a]	238.8 ± 16.48*[a]	197.8 ± 48.3*[a]	229.8 ± 15.04*[ab]
D + AE (200 mg/kg)	93.6 ± 6.16	394 ± 10.21*[ab]	125.4 ± 6.79*[a]	112.2 ± 4.79[ab]	96.8 ± 8.17[ab]	129.8 ± 9.44*[ab]
D + AE (400 mg/kg)	91.6 ± 9.51	417.4 ± 18.84*[ab]	196.6 ± 6.35*[a]	143.8 ± 5.07*[ab]	105.8 ± 7.32[ab]	198.6 ± 35.62*[a]
D + M	74 ± 4.722	215.8 ± 11.28*	205.6 ± 24.15*	180.6 ± 7.59*	169.4 ± 5.39*	167.2 ± 8.46*

*, a, b. The mean difference is significant at the 0.05 level (2-tailed).
Dunnett t-tests treat one group as a control, and compare all other groups against it.
Group C, DNT, D + EE (200 mg/kg), D + EE(400 mg/kg), D + ME (200 mg/kg), D + ME (400 mg/kg), D + AFE (200 mg/kg), D + AFE (400 mg/kg), D + EAE (200 mg/kg), D + EAE (400 mg/kg), D + AE (200 mg/kg), D + AE (400 mg/kg), D + M represent Control, Diabetic non-treated, Diabetic + ethanol extract (200 mg/kg), Diabetic + ethanol extract (400 mg/kg), Diabetic + methanol extract (200 mg/kg), Diabetic + methanol extract (400 mg/kg), Diabetic + acid fraction extract (200 mg/kg), Diabetic + acid fraction extract(400 mg/kg), Diabetic + ethyl acetate extract (200 mg/kg), Diabetic + ethyl acetate extract (400 mg/kg), Diabetic + acetone extract (200 mg/kg), Diabetic + acetone extract (400 mg/kg), and Diabetic + Metformin (reference drug) treated diabetic rats respectively.
< 0.05 indicates a significant difference when compared to the control group.
a< 0.05 indicates a significant difference when compared to the diabetic non-treated group.
b < 0.05 indicates a significant difference when compared to the diabetic treated with Metformin (reference drug) group.

Table 6
Changes on Cytokines, antioxidants and insulin secretion after oral administration of different *Boswellia.sacra* extracts on diabetic rats

	IL-2 (pg/ml)	IL-8 (pg/ml)	GSH (μg/ml)	SOD (IU/ml)	Serum Insulin (ng/ml)
C	143.91 ± 13.58[a]	39.69 ± 2.52[a]	4.28 ± 0.34[a]	12.91 ± 0.63[a]	1.31 ± 0.1[a]
DNT	448.51 ± 44.61*	102.99 ± 8.95*	2.46 ± 0.07*	4.04 ± 0.28*	0.65 ± 0.13*
D + EE (200 mg/kg)	157.94 ± 13.42[a]	45.748 ± 1.39[a]	4.16 ± 0.10[a]	10.22 ± 0.13[a]	1.28 ± 0.3[a]
D + EE (400 mg/kg)	160.05 ± 16.93[a]	50.49 ± 1.36[a]	3.98 ± 0.21[a]	11.56 ± 0.26[a]	1.08 ± 0.04[a]
D + ME (200 mg/kg)	181.32 ± 22.55[a]	46.79 ± 4.25[a]	3.72 ± 0.11[a]	9.68 ± 0.2[a]	1.06 ± 0.0[a]
D + ME (400 mg/kg)	204.77 ± 6.97[a]	50.08 ± 4.55[a]	3.85 ± 0.32[a]	10.51 ± 0.26	0.99 ± 0.01[a]
D + AFE (200 mg/kg)	197.03 ± 15.67[a]	46.82 ± 3.75[a]	3.19 ± 0.21	8.79 ± 0.61[a]	1.08 ± 0.05[a]
D + AFE (400 mg/kg)	220.23 ± 17.19[a]	50.05 ± 3.29[a]	2.91 ± 0.51	9.15 ± 0.27[a]	1.21 ± 0.07[a]
D + EAE (200 mg/kg)	191.32 ± 12.48[a]	40.09 ± 2.26[a]	3.76 ± 0.30[a]	8.55 ± 0.92[a]	1.17 ± 0.04[a]
D + EAE (400 mg/kg)	217.34 ± 20.34[a]	47.58 ± 1.42[a]	3.83 ± 0.27[a]	11.59 ± 0.31[a]	1.06 ± 0.09[a]
D + AE (200 mg/kg)	185.65 ± 21.78[a]	43.62 ± 2.59[ш]	3.77 ± 0.27[a]	7.27 ± 0.46[a]	0.98 ± 0.05[a]
D + AE (400 mg/kg)	235.15 ± 17.25[a]	51.99 ± 4.05[a]	3.65 ± 0.28[a]	8.59 ± 0.36[a]	0.91 ± 0.09[a]
D + M	162.45 ± 23.2[a]*	45.11 ± 6.22[a]	3.89 ± 0.12[a]	10.07 ± 0.51[a]	1.19 ± 0.05[a]

Group C, DNT, D + EE (200 mg/kg), D + EE(400 mg/kg), D + ME (200 mg/kg), D + ME (400 mg/kg), D + AFE (200 mg/kg), D + AFE (400 mg/kg), D + EAE (200 mg/kg), D + EAE (400 mg/kg), D + AE (200 mg/kg), D + AE (400 mg/kg), D + M represent Control, Diabetic non-treated, Diabetic + ethanol extract (200 mg/kg), Diabetic + ethanol extract (400 mg/kg), Diabetic + methanol extract (200 mg/kg), Diabetic + methanol extract (400 mg/kg), Diabetic + acid fraction extract (200 mg/kg), Diabetic + acid fraction extract(400 mg/kg), Diabetic + ethyl acetate extract (200 mg/kg), Diabetic + ethyl acetate extract (400 mg/kg), Diabetic + acetone extract (200 mg/kg), Diabetic + acetone extract (400 mg/kg), and Diabetic + Metformin (reference drug) treated diabetic rats respectively.
< 0.05 indicates a significant difference when compared to the control.
[a] < 0.05 indicates a significant difference when compared to the diabetic.

diabetic rats treated with metformin. Conversely, oral administration of *B. sacra* extracts to diabetic rats at the two dosages employed for 3 weeks decreased oxidative stress, as evidenced by a significant ($p < 0.05$) increase in SOD and GSH compared to the diabetic untreated group.

Furthermore, blood insulin levels in diabetic non-treated rats were significantly ($p < 0.05$) lower than those in either control or diabetic rats treated with metformin. The oral administration of *B. sacra* extracts at two dosages to diabetic rats elevated blood insulin levels significantly ($p < 0.05$) compared to normal plasma rat levels.

3.6. Histopathological investigation

Fig. 2A (P1– P8) depicts a representative microscopic examination of thin sections of pancreatic rat tissues treated with various *B. sacra* extracts and stained with hematoxylin-eosin. The control group's pancreas shows a normal cellular composition in the pancreatic islets of

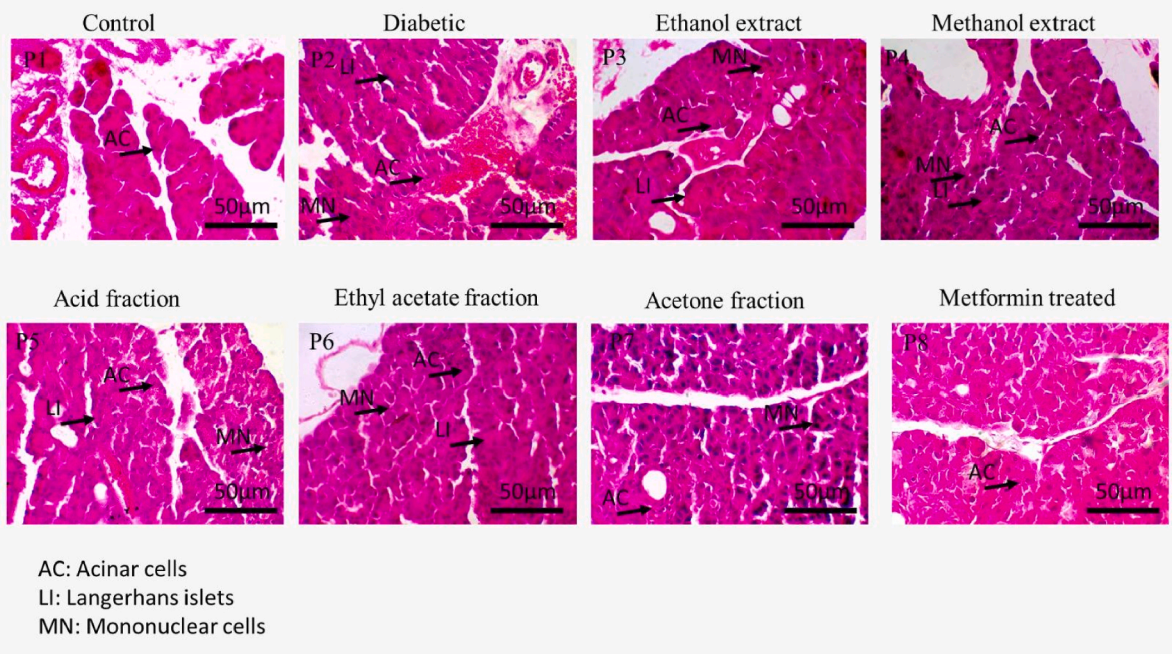

Fig. 2A. (P–P8): (Color) Representative photographs of H&E-stained thin sections of rat pancreas, X400: (P1): Normal control rat pancreas with normal histological findings in pancreatic acinar cells; (P2) STZ-induced diabetic rat pancreas with islet endocrine cell damage and necrosis; Langerhans with islet shrinkage, degenerated acini cells, and islet of Langerhans. (P3–P7) Pancreas sections from rats given extracts at 200 or 400 mg/kg dosages after streptozotocin-induced diabetes: showing regeneration with restoration of Langerhans islets and pancreatic cells. (P8) pancreas of metformin-treated rat pancreas with a mild interstitial inflammatory cell infiltrate associated with acinar hyperplasia.

Langerhans as well as normal histological findings in pancreatic acinar cells (P1). Meanwhile, the diabetic rat pancreas had severe cell damage, necrosis, and inflammation, as well as islet shrinkage, degenerated acini cells, and Langerhans islets (P2).

Following streptozotocin-induced diabetes, rats were treated with 200 mg/kg or 400 mg/kg of different *B. sacra* extracts (P3–P8) showed

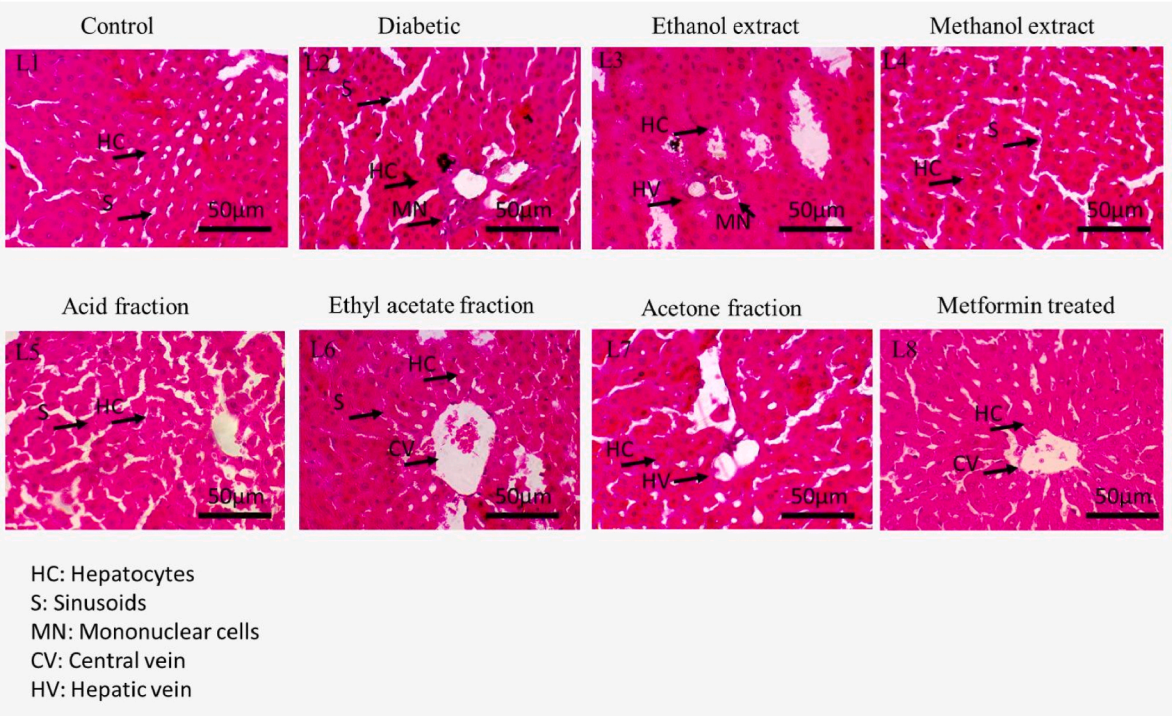

Fig. 2B. (L1-L8): (Color) Representative photographs of H&E-stained thin sections of rat liver, X400: (L1) Normal control of rat liver section demonstrating normal histological findings in the parenchymal cells of the liver; (L2) streptozotocin-induced diabetic rat liver section: demonstrating variable degree of bridging necrosis of hepatocytes, most prominent in centrilobular and mononuclear cellular infiltrate in the lobule. (L3-L7) After streptozotocin-induced diabetes, liver sections from rats treated with 200 or 400 mg/kg extracts exhibited centrilobular regeneration with restoration of the central vein, vacuole, sinusoidal spaces, and hepatocytes with mild necrosis. (L8) Metformin-treated rat liver with mild infiltration of inflammatory cells associated with steatosis.

significant improvement in cellular architecture as measured by regeneration with restoration of normal islet and pancreatic cell population size, with *B. sacra* ethanol extract showing the most improvement (P3).

Pancreatic cells from metformin-treated hyperglycemic rats showed a mild interstitial inflammatory cell infiltrate associated with acinar hyperplasia (P8).

The histopathological effect of diabetic, diabetic *B. sacra* treated with different extracts, and diabetic metformin-treated rats on liver tissue was observed when compared to control rats (Fig. 2B; L1-L8).

Normal control rat liver section demonstrating normal histological findings in the liver's parenchymal cells. Diabetic rat liver sections revealed some histopathological abnormalities, including variable degrees of bridging necrosis of hepatocytes, most prominent in centrilobular and mononuclear cellular infiltrates in the lobule, hepatocyte shrinkage, and hepatocyte fragmentation (apoptosis). These findings, however, were not detected in rats given 200 or 400 mg/kg of different *B. sacra* extracts (L3-L8). Following streptozotocin-induced diabetes, liver sections from rats treated with different extracts of *B. sacra* demonstrated centrilobular regeneration, with the restoration of the central vein, vacuole, sinusoidal spaces, and hepatocytes with mild necrosis. In the ethanol extract, the regeneration was more noticeable (L3). Metformin treatment resulted in a mild infiltration of inflammatory cells associated with steatosis in the rat liver (L8).

4. Discussion

Diabetes is a chronic and serious disease with serious consequences for mortality and health. A search for more effective treatments is currently underway. In line with this, diabetic patients all over the world rely heavily on traditional medicines to treat diabetes and its complications [19].

Indian frankincense (*B. serrata*) gum resin and its extracts are the only species that have been fully researched for their antihyperglycemic and antioxidant effects [11,12]. In contrast, little effort was made to study the same phenomenon in the other 25 *Boswellia* species, including the Omani species *B. sacra*. The goal of this study is to investigate into the hypoglycemic, anti-inflammatory, and antioxidant effects of different Omai *B. sacra* extracts in diabetic rats that had been exposed to STZ for 29 days and compare them to metformin, the standard treatment for type 2 diabetes.

Diabetes mellitus, according to Hashemnia et al. [20], is a collection of illnesses distinguished by insulin sensitivity and/or hyposecretion. Even though insulin is the most powerful drug known to medicine, scientists have been looking for plant-derived compounds with properties similar to insulin to treat diabetes [21].

The use of herbal drugs for therapeutic purposes and as food supplements has led to an increase in adverse reactions and toxicity that is occurring globally. Previous reports have stated that boswellia species are safe. However, there are limited studies on the organ toxicity of *B. sacra*. For example, Al-Yahya et al. [22] found that the methanolic extract of *B. sacra* oleo gum resin is safe up to oral doses of 1000 mg/kg, with no noticeable effects on the kidney or liver after 28 days of administration. Rashan and Suleiman [23] recently conducted acute and chronic oral toxicity testing on extracts derived from Omani *B. sacra* gum resin using OECD test guideline 408. For acute toxicity, they reported that ethanol *B. sacra* acid extract was used twice a day for 14 days at a dose of 2000 mg/kg body weight, and based on their observations, they concluded that *B. sacra* extract is classified as 'Category 5 or unclassified' with an LD50 greater than 2000 mg/kg. However, in the chronic toxicity test, they discovered that repeated doses of ethanol *B. sacra* acid extract for 90 days revealed that *Boswellia* extract was safe at the highest dose tested, i.e., 1000 mg/kg. Furthermore, body weight gain, feed intake, and clinical signs of toxicity were all within normal limits when compared to normal rats. In addition, the clinical chemistry and histopathological parameters tested revealed that the extract had no toxicity in any of the organs or major biochemical metabolites. When you consider how much extract people consume, previous studies have reported quite high dosages. The extract is advertised for human consumption at doses of up to 1000 mg per day, or around 200 mg/kg in rats. The maximum dosage investigated in previous studies was nearly five times that of the highest dose administered to humans. As a result, the *B. sacra* gum resin extracts utilized in the current investigation at concentrations of 200 and 400 mg/kg were relatively safe. Metformin has become the most commonly prescribed medicine for type 2 diabetes (T2DM) due to its excellent blood glucose management abilities and superior safety profile [24]. Furthermore, the American Diabetes Association and the European Association for the Study of Diabetes recommended metformin as the first-line treatment for T2DM in their 2012 guidelines [24].

Metformin's primary benefit is its ability to gradually reduce blood glucose levels [25]. This is followed by an increase in peripheral tissue insulin sensitivity that does not increase the risk of hypoglycemia [26] or weight gain [27]. Previous research has suggested that metformin may have a range of functional profiles, including anti-inflammatory, anti-cancer, and cardiovascular protection [28]. As a result, in the current study, we used metformin (250 mg/kg body weight per day) as a standard hypoglycemic medicine. The dosage was chosen based on regulatory standards and previous study findings [29]. In this study, we evaluated the potential therapeutic benefits of *B. sacra* extracts on rat streptozotocin (STZ)-induced hyperglycemia. The HPLC-MS/MS analysis used in this study revealed a significant variation in the PTA contents of *B. sacra* from Oman when different extracts were used. In all extracts studied, *B. sacra* oleo-gum resins contain a high proportion of acetyl-β-boswellic acid (β-ABA) and acetyl-11-keto-β-boswellic acid (AKBA), among other PTA components. In terms of anti-diabetic potential, the ethanol extract contains the highest levels of PTA of the two major boswellic acids, β-ABA and AKBA.

The maximum concentrations of β-ABA and AKBA in the oleo-gum resins of *B. sacra* extracted with ethanol reported in this study are 111 and 73.2 mg/g (w/w), respectively. This is higher than what Syrovets et al. [30] reported for β-ABA and AKBA of the species *B. dalzielli* (32.5 and 64.7 mg/g) and Schmiech et al. [16] recorded for the species *B. sacra* (40.9 and 31.3 mg/g).

In this study, we found that after 7 days of oral administration of different *B. Sacra* extracts to diabetic groups, blood glucose concentrations were lower than in the diabetic group that received no treatment. Furthermore, diabetic rats given a 200 mg/kg/day ethanol extract of *B. Sacra* had lower blood glucose levels than diabetic rats given no treatment, diabetic rats given other extracts, and diabetic rats given metformin, with blood glucose concentrations comparable to control rat levels. Based on these results, Namjou and Rouhi-Broujent [12] found that diabetic rats fed *B. serrata* extract had lower glucose, liver enzyme, kidney, and lipid profiles than diabetic control rats. Helal et al. [14] discovered that treating diabetic rats with *B. carteri* improved hypoglycemia significantly.

The extract of *B. sacra* oleo-gum resin contains a high concentration of β-ABA and AKBA, which have antihyperglycemic properties via a variety of mechanisms. It was originally thought that AKBA's ability to stimulate the AMP-activated protein kinase (AMPK) pathway was responsible for its antihyperglycemic effects.

The metabolic sensor AMPK regulates cellular energy balance by increasing ATP generation via catabolic pathways while inhibiting ATP breakdown via anabolic pathways. Numerous health advantages, such as enhanced fatty acid oxidation, reduced inflammation, and enhanced glucose uptake and utilization in skeletal muscle cells, have been associated with AMPK activation [31].

Furthermore, Mahdian et al. [32] found that *B. sacra* may increase insulin sensitivity and glucose uptake by cells, which may contribute to its blood glucose-lowering effects. The precise mechanism underlying this is unknown and requires further investigation.

In addition, Azemi et al. [11] discovered that *B. serrata* extracts at a dose of 200 mg/kg had the greatest impact on reducing HbA1c and

blood sugar levels in diabetic rats when compared to other higher doses in their studies.

It is crucial to stress that our findings are consistent with previous studies in that a lower dose of *B. sacra* ethanol extract, 200 mg/kg/day, lowers blood sugar levels more effectively than a higher dose, 400 mg/kg/day, which may be related to the hormesis phenomenon [33]. As a result, *B. sacra* extract may follow a hormetic response, in which lower concentrations stimulate positive adaptive responses in the body while higher concentrations may induce negative or counterproductive effects. Furthermore, the bioavailability of active compounds in *B. sacra* extract may vary depending on concentration. Higher concentrations may overwhelm the body's absorption and metabolic capacity, resulting in decreased bioavailability and therapeutic effects. Lower concentrations, on the other hand, may allow for better absorption and distribution of the active components [34].

Other options include *B. sacra* extract, which contains several bioactive compounds, including boswellic acids, terpenes, and flavonoids, which may have synergistic effects at certain concentrations. Lower concentrations may allow for a more balanced interaction between these compounds, maximizing their combined therapeutic potential. Alternatively, the efficacy of *B. sacra* extract may be related to its interaction with specific cellular receptors and signaling pathways. Lower concentrations may stimulate these receptors and signaling cascades more effectively, resulting in improved physiological responses. In contrast, higher concentrations may saturate or overwhelm these receptors, resulting in diminished or altered effects. It is important to note that these hypotheses are speculative and necessitate additional scientific research to establish their validity.

Diabetes is associated with significant weight loss. We noticed that diabetic control (untreated) rats lost weight throughout the experiment (29 days). During this time, they lost 27.9% of their initial body weight. This could be attributed to increased muscle atrophy and tissue protein loss [35]. On the other hand, diabetes-mediated body weight loss was reversed by treatment with *B. sacra* ethanol extract (200 mg/kg), resulting in a considerable improvement in body weight (Table 4). Likewise, Khan et al. [36] demonstrated that β-BA and β-KBA reversed body weight reduction in diabetic-treated rats, resulting in significant weight recovery. This weight gain was matched to that of rats receiving metformin treatment. This resumption of body weight loss could be related to *B. sacra's* hypoglycemic actions and, as a result, to its protective effect on tissue structural components.

Streptozotocin has been routinely used in animal experiments to induce diabetes due to its damaging effects on pancreatic β-cells [37]. STZ's cytotoxic action is associated with the production of reactive oxygen species (ROS), which causes oxidative damage. STZ's cytotoxic action is due to the suppression of a free radical scavenging enzyme, which increases superoxide radical production and hence potentially injures pancreatic β-cells [38].

Additionally, STZ's intracellular metabolism generates nitric oxide, which causes DNA fragmentation and necrosis in β-cells. As a result, the rate of insulin synthesis slows, resulting in hyperglycemia as a clinical issue [39]. In comparison to control rats, diabetic rats in this study had higher glucose and lower insulin levels. Nonetheless, diabetic rats treated orally for three weeks with various *B. sacra* extracts showed reduced blood glucose and increased insulin levels in the STZ-treated diabetic rats. This may be explained by the fact that *B. sacra* increases peripheral glucose uptake while inhibiting hepatic gluconeogenesis or intestinal glucose absorption via insulin secretion stimulation.

SOD shields tissues against oxygen-induced free radicals by catalyzing the elimination of superoxide radicals, which are transformed into H_2O_2 and molecular oxygen, both of which are harmful to cell membranes and other biological components [40]. Previous research found that STZ caused beta cells to produce reactive oxygen free radicals, which are thought to inactivate and reduce SOD and other antioxidative enzyme activities. These antioxidant enzymes are necessary to avoid oxidative cell damage [41]. Furthermore, in most mammalian cells, GSH is the most important antioxidant. GSH is an important intracellular redox buffer that serves as a cofactor for a variety of enzymes, a direct free radical scavenger, and a substance for glutathione peroxidase activity. It also aids in the maintenance of the active sites of exogenous antioxidants such as vitamins E and C [42].

In the current investigation, GSH and SOD levels in diabetic rat plasma were found to be lower than those in control or metformin- or extract-treated diabetic rats. Kherouf et al. [43] recently reported a comparable change in antioxidant status, notably a decrease in glutathione (GSH), in rats exposed to STZ.

Different *B. sacra* extracts administered orally to diabetic rats effectively recovered the altered levels of these antioxidants, possibly due to their scavenging properties, demonstrating that *B. sacra* content has antioxidant potential. Flavonoids, according to Rajadurai and Prince [44], prevent changes in mitochondrial lipid peroxides, which are oxidative defense enzymes similar to SOD in rodents.

The mechanism by which *B. sacra* extract increases SOD activity is unknown, but it could be related to its high terpenoids and phenolic compound content, as ethanol extract had the highest AKBA concentration in this study when compared to the other extracts. These compounds have been shown to have potent antioxidant properties and to improve the activity of antioxidant enzymes. Chen et al. [45] discovered that AKBA increased SOD activity while decreasing MDA and ROS, lending support to our hypothesis.

Microscopic, thin sections of the pancreas and liver from diabetic, non-treated rats were used to further demonstrate the antioxidant activity. These sections revealed extensive damage and inflammation with loss of normal architecture of pancreatic β-cells (Fig. 2A P2) and a variable degree of bridging necrosis of hepatocytes, most prominent in the centrilobular and mononuclear cellular infiltrate in the lobule (Fig. 2B L2). In contrast, 200 and 400 mg/kg of *B. sacra* extracts administered orally restored FBG and insulin levels, as evidenced by the regeneration of β-cells and preservation of pancreatic morphology (Fig. 2A P3-7) and liver regeneration of the central vein, vacuole, and sinusoidal spaces (Fig. 2B L3-7), thereby significantly reducing the harmful effects of diabetes.

A growing body of evidence suggests a link between inflammatory processes, subsequent ***β***-cell dysfunction, and impaired insulin signaling [46]. Our findings show that diabetic rats have significantly higher levels of IL-2 and IL-8 compared to non-diabetic rats, which is consistent with previous studies that examined circulating IL-8 concentrations in the presence of diabetes, finding higher IL-8 levels in people with type 2 diabetes mellitus [47].

Shehata et al. [48] observed significant increases in pro-and anti--inflammatory cytokines (including IL-2) in the blood of rats treated with multiple low-dose streptozotocin (MLD-STZ) for 5 days after the last STZ injection. On the other hand, Dogan et al. [49] revealed that decreased IL-2 synthesis is exclusive to insulin-dependent diabetes mellitus (IDDM).

This study found that different *B. sacra* extracts have anti-inflammatory effects since they reduce the levels of IL-2 and IL-8 produced by STZ-induced diabetic rats. Similar to our findings, Shehata et al. [48] noted that 11-keto-boswellic acid (KBA) or O-acetyl-11-keto-boswellic acid (AKBA) and extracts from the gum resin of *B. serrata* reduce cytokines (pro- and anti-inflammatory) in the blood, resulting in lower hyperglycemia and prevention of islet destruction in a diabetic animal. This protection is most likely due to the ability of boswellic acid to inhibit 5-lipoxygenase, an enzyme involved in the production of leukotrienes known to promote inflammation and thus cytokine inhibition, which causes islet inflammation [50,51].

The current study found that *B. sacra* has anti-inflammatory, anti-diabetic, and anti-oxidant activities. These characteristics are primarily due to the TPA's substances, notably ABA and AKBA. This result is comparable to that of Schmiech et al. [16] who discovered a link between PTA levels in *Boswellia* extracts and cytotoxic activity against cancer cells.

5. Conclusion

The two most abundant compounds in *B. sacra* oleo gum resin extracts were β-ABA and AKBA, with the ethanolic extract having the highest total PTA content. All diabetic rats given a 200 mg/kg/day ethanol extract of *B. sacra* had lower blood glucose levels and higher blood insulin levels than all other groups (diabetic non-treated groups, diabetic-treated groups with other extracts, or diabetic-treated rats with metformin), with blood glucose levels comparable to control rats. Oral administration of *B. sacra* extracts (two doses) to diabetic rats for three weeks reduces serum IL-2 and IL-8 levels, as well as oxidative stress, as evidenced by higher SOD and GSH levels compared to the diabetic untreated group. The ethanol extract was the most effective in improving centrilobular regeneration and the structure of Langerhans islets and pancreatic cells in rats treated with different B. sacra extracts (two doses each). As a result, *B. sacra* may have therapeutic potential for diabetes prevention. Its antioxidant properties may be responsible for its antidiabetic actions, or it may prevent the production or activity of cytokines that are required to trigger islet inflammation in response to an autoimmune disease.

CRediT author statement

Conceptualization, Methodology and Supervison: Al-Matubsi, Rashan, and Aburayyan, Data curation: Aburayyan, and Abuarqoub, Writing- Original draft preparation: Al-Matubsi, and Rashan, Formal analysis: Al-Matubsi, Rashan, Aburayyan, and Al Hanbali, Investigation: Al-Matubsi, Rashan, Rashan, Efferth, and Aburayyan, Validation: Al-Matubsi and Aburayyan. Reviewing final draft: Al-Matubsi, Rashan, Aburayyan, Al Hanbali, Abuarqoub, and Efferth, Project administration: Al-Matubsi. The final manuscript was reviewed and approved by all authors. All authors agree to bear full responsibility for the integrity and accuracy of their work.

Declaration of generative AI in scientific writing

The authors confirm that no generative AI or AI-assisted technologies were used in the writing process.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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