Effect of lemon balm (*Melissa officinalis*) aqueous extract on streptozotocin-induced diabetic rats

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Background
Lemon balm (*Melissa officinalis*) has a significant role in curing diseases and maintaining health through its antioxidant capacity. The aim of this study was to evaluate antidiabetic effect of lemon balm aqueous extract (LBAE) on streptozotocin (STZ)-induced diabetic rats.
Materials and methods
The extract was administered to STZ-induced diabetic rats in low and high doses (200 and 400 mg/kg body weight/day, respectively) for 4 weeks. Serum insulin, glucose, lipid profiles, alkaline phosphatase, serum alanine aminotransferase, aspartate transaminase, creatinine and urea levels were determined, whereas total antioxidant capacity, malondialdehyde, nitric oxide, Na^+/K^+ ATPase activity (ATPase), tumor necrosis factor-α, and cluster of differentiation 4 levels were evaluated in liver and kidney tissue homogenates.
Results and conclusion
Oral administration of LBAE significantly decreased glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol, malondialdehyde, nitric oxide, tumor necrosis factor-α, and cluster of differentiation 4 levels. However, insulin, high-density lipoprotein cholesterol, and total antioxidant capacity levels significantly increased with respect to diabetic control group. These findings revealed that LBAE possesses antihyperglycemic and antihyperlipidemic effects against STZ-induced disorders in diabetic rats. Hence, it can be used in the management of diabetes mellitus.

Keywords:
diabetes, *Melissa officinalis*, rats, streptozotocin

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Introduction

The occurrence of diabetes mellitus elevated annually all over the world. Number of diabetic patients will increase to 592 million in 2035 [1]. Insulin-dependent patients are the majority, whereas noninsulin-dependent patients are small proportions (7–10%) of diabetic patients [2]. Type 1 diabetes (T1D), a chronic disease, is produced via pancreas autoimmune destruction of β-cells leading to hyperglycemia and insulin deficiency [3]. Thus, disturbances may be generated in glucose and lipid homeostasis resulting in dyslipidemia and hyperglycemia [4] causing increased production of oxygen free radicals (FRs) as a result of autoxidation of glucose [5] and glycosylation of protein [6] leading to oxidative stress, which is associated with several health complications, including antipathies, cardiovascular disorders, blindness, renal failure, neuropathies, and cancers [7].

Streptozotocin (STZ) is an antibiotic, used experimentally owing to its ability to induce insulin-dependent diabetes mellitus following multiple low-dose (30–50 mg/kg) injection. STZ-treated rats developed clinical features and signs that are similar to those found in T1D mellitus [8].

Plants provide a natural way for treating diverse complex disorders [9]. Edible biomaterial extracts have become a major focus of nutritional research to develop healthy and safe nutraceutical functional foods [10]. Recently, drug formulation from natural herbs attracted the attention of many researchers [11].

Lemon balm, Lamiacea family, has beneficial and flavouring properties in food and cosmetics cultivated not only for its characteristic lemon-scented leaves but also for several purposes. Balm has therapeutic properties in nervous agitation and gastrointestinal complaints [12]. Rosmarinic acid which is the main phenolic acid compound found in the leaves is responsible for the antioxidative and

antiviral activities of plant extract [13]. This study aimed to investigate the antidiabetic effects of lemon balm aqueous extract (LBAE) on STZ-induced diabetic rats.

Materials and methods

Animals

Forty Wistar rats were used. The animals were 6–10 weeks old (150–200 g). Animals were kept in clean plastic cages and housed in the animal holdings of the National Research Centre, Egypt. The animals were exposed to 12-h light, 12-h darkness cycle at room temperature. They were maintained on animal feeds and allowed free access to water and feeds. All animal methods are in accordance with the recommendations stated by ethics committee of the National Research Centre approval on animal care [14].

Drugs and chemicals

STZ was purchased from Sigma-Aldrich Corporation is an American chemical, life science and biotechnology company (St. Louis, Missouri, United States).

Glimepiride (Amaryl) was purchased from Sanofi-Aventis (Cairo, Egypt) El Sawah El Amiriya.

Plant extraction

Overall, 100 g of the powdered herb material was placed in a 1000-ml round-bottom quick-fit flask, and 400 ml distilled water was added. The mixture was left for 24 h. Water fractions were combined and filtered through qualitative no. 1 Whatman filter paper (Whatman International Ltd, Maidstone, UK). In Aroma and Flavoring Department, National Research Centre, the filtrate was subjected to lypholyzation process through freeze drier (Snijders Scientific, Tilburg, Holland) under pressure 0.1–0.5 mbar and temperature −35 to −41°C conditions. The dry extract was stored at −20°C until used.

Induction of diabetes

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of a freshly buffered (0.1 mol/l citrate, pH 4.5) solution of STZ at a dosage of 50 mg/kg body weight [15]. After 72 h of STZ administration, the tail vein blood was collected to determine fasting blood glucose level with an Accu Chek sensor comfort glucometer (China). Only rats with hyperglycemia (glucose over 250 mg/dl) were considered as diabetic and included in the experiment [16].

Experimental design

Rats were divided randomly into five groups (n=8) and treated orally for the experimental period of 4 weeks as follows: group 1, normal control animals; group 2, diabetic group that received STZ only; group 3, diabetic group that received Amaryl (0.1 mg/kg body weight/day) orally [17]; and groups 4 and 5, diabetic groups that received LBAE (200 and 400 mg/kg body weight/day) orally [18].

Serum and tissue biochemical analysis

At the end of the experiment, overnight fasting animals were Ether anesthetized. Venous retro orbital blood samples were collected using a glass capillary without anticoagulant. Serum was separated by centrifugation at 3000 rpm/min for 15 min. The resulting samples were stored at −20°C until assayed. Serum was used for estimation of glucose, insulin, lipid profiles, alanine aminotransferase, aspartate transferase, alkaline phosphatase, urea, and creatinine using specific diagnostic kits (Biodiagnostic, Dokki, Giza, Egypt).

Livers and kidneys were removed and washed in ice-cold saline solution immediately, and then each organ was homogenized in 0.1 mol/l potassium phosphate buffer (pH 7.4) using Tissue master TM125 (Omni International, Kennesaw United States). After centrifugation at 3000 r/min for 10 min, the clear supernatant was stored at −80°C to be used for estimation of total antioxidant capacity (TAC), malondialdehyde (MDA), nitric oxide (NO), Na^+-K^+-ATPase using specific diagnostic kits (Biodiagnostic), tumor necrosis factor-α (TNF-α), and cluster of differentiation 4 (CD4) levels using Elabscience Biotechnology Inc. Houston, Texas, United States.

Statistical analysis

All the values are presented as mean±SE. Comparisons between different groups were carried out using one-way analysis of variance followed by least significant difference (LSD) test for multiple comparisons. GraphPad Prism software, version 5 (GraphPad Software Inc., San Diego, California, USA), was used to carry out these statistical tests. The difference was considered significant when P less than 0.05.

Results

Effect of *Melissa officinalis* aqueous extract on serum glucose and insulin levels

The glucose level was increased whereas insulin level was decreased in diabetic rats compared with normal

rats. These results were reversed in diabetic group that received Amaryl when compared with diabetic rats. However, treatment with both doses of LBAE reduced the increased level of blood glucose and increased level of serum insulin when compared with diabetic rats) Table 1).

Effect of *Melissa officinalis* aqueous extract on serum lipid profile

Table 2 shows that rats in diabetic group displayed significantly elevated triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL) levels in comparison with normal group. However, serum high-density lipoprotein (HDL) level of diabetic rats was significantly lower than that of normal ones. In contrast, diabetic rats received Amaryl as well as those treated with LBAE in both low and high doses, showed significant decreases in TG, TC, and LDL levels and a significant increase in HDL level when compared with diabetic rats.

Table 1 Serum insulin and blood glucose levels in normal, streptozotocin-induced diabetic Amaryl-treated and streptozotocin-induced diabetic lemon balm aqueous extract-treated rats

Groups	Insulin (μIU/ml)	Glucose (mg/dl)
Normal control	48.23±1.2	119±2.4
Diabetic control	20.46±1.3[a]	304±5.9[a]
Amaryl (0.1 mg/kg)	41.03±5.5[b]	156±3.1[a,b]
LBAE (200 mg/kg)	50.29±2.5[b]	127±2.1[b]
LBAE (400 mg/kg)	45.03±1.6[b]	123±11.2[b]

Data were expressed as mean±SE (n=8). Statistical analysis was carried out by one-way analysis of variance, $P<0.05$; followed by LSD test for multiple comparisons. LBAE, lemon balm aqueous extract. [a]$P<0.05$, significant from normal control. [b]$P<0.05$, significant from diabetic control.

Effect of *Melissa officinalis* aqueous extract on liver and kidney functions

Table 3 represents liver and kidney biochemical parameters. Significant alterations in these parameters were noticed in STZ diabetic rats as compared with normal rats. In contrary, STZ diabetic rats that received Amaryl reduced these elevations. The treatment with LBAE significantly improved the levels of these biochemical markers toward the normal values.

Effect of *Melissa officinalis* aqueous extract on oxidative stress markers of liver and kidney

Table 4 reveals that diabetic animals exhibited significant increases in MDA and NO levels concomitant with significant decreases in TAC level in both liver and kidney homogenates when were compared with normal control. On the contrary, administration of Amaryl and LBEA to diabetic rats succeeded to decreases in MDA and NO levels in concomitant with significant increases in TAC level in both liver and kidney homogenates as compared with diabetic rats.

Effect of *Melissa officinalis* aqueous extract on cluster of differentiation 4 and tumor necrosis factor-α levels

Liver and kidney CD4 levels were lower, whereas TNF-α levels were higher in diabetic rats compared with normal rats. However, treatment of diabetes rats

Table 2 Serum lipid profiles in normal, streptozotocin-induced diabetic Amaryl-treated and streptozotocin-induced diabetic lemon balm aqueous extract-treated rats

Groups	TG (mg/dl)	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal control	146±3.15	119±6.85	66.6±4.25	85.2±4.36
Diabetic control	307±18.37[a]	445±3.85[a]	154±6.26[a]	44.9±4.20[a]
Amaryl (0.1 mg/kg)	105±1.15[a,b]	105±5.19[a,b]	98±3.11[a,b]	60±3.64[a,b]
LBAE (200 mg/kg)	145±3.73[b]	122±0.21[b]	89±6.23[a,b]	69.9±2.49[a,b]
LBAE (400 mg/kg)	122±2.55[b]	109±2.83[b]	79.8±5.61[b]	74±5.04[b]

Data were expressed as mean±SE (n=8). Statistical analysis was carried out by one-way analysis of variance, $P<0.05$; followed by LSD test for multiple comparisons. HDL, high-density lipoprotein; LBAE, lemon balm aqueous extract; LDL, low-density lipoprotein. [a]$P<0.05$, significant from normal control. [b]$P<0.05$, significant from diabetic control.

Table 3 Serum liver and kidney functions in normal, streptozotocin-induced diabetic Amaryl-treated, and streptozotocin-induced diabetic lemon balm aqueous extract-treated rats

Groups	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	Urea (mg/dl)	Creatinine (mg/dl)
Normal control	146±3.1	25.8±0.4	269±6.1	7.13±0.27	0.54±0.31
Diabetic control	307±18.3[a]	30.4±0.5a	584±1.2[a]	11.74±1.02[a]	0.78±0.42[a]
Amaryl (0.1 mg/kg)	105±1.1[a,b]	28±0.6[a,b]	404±6.6[a,b]	7.15±0.41[b]	0.71±0.25[a,b]
LBAE (200 mg/kg)	145±3.7[b]	27.2±0.3[b]	576±0.4[a]	8.72±0.35[b]	0.68±0.13[b]
LBAE (400 mg/kg)	122±2.5[b]	27.5±0.3[b]	569±2.8[a]	7.90±0.13[b]	0.66±0.27[b]

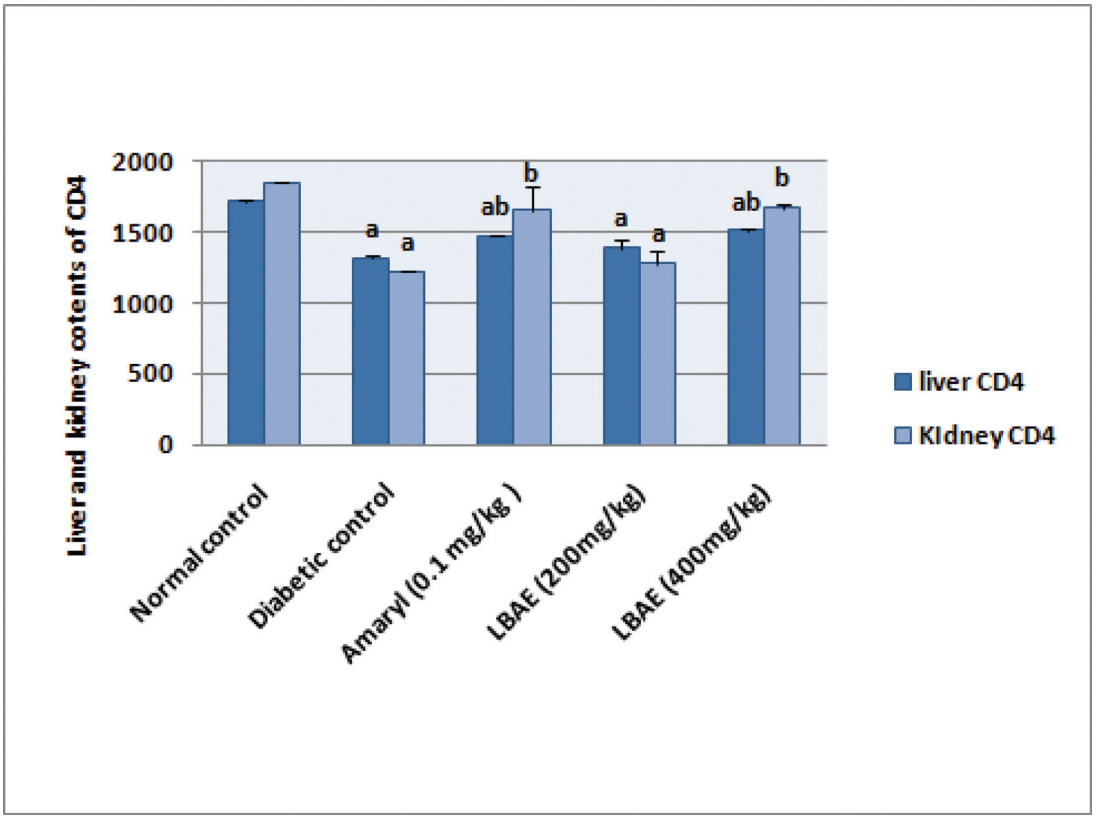
Data were expressed as mean±SE (n=8). Statistical analysis was carried out by one-way analysis of variance, $P<0.05$; followed by LSD test for multiple comparisons. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; LBAE, lemon balm aqueous extract. [a]$P<0.05$, significant from normal control. [b]$P<0.05$, significant from diabetic control.

Table 4 Oxidative stress markers of liver and kidney in normal, streptozotocin-induced diabetic Amaryl-treated, and streptozotocin-induced diabetic lemon balm aqueous extract-treated rats

Groups	MDA (nmol/g)		NO (nmol/g)		TAC (μmol/g)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal control	917±20.1	726±9.1	32.2±0.3	29.8±0.4	527±12.1	863±15.7
Diabetic control	1129±24.8[a]	932±11.6[a]	41.1±0.5[a]	40.3±0.5[a]	417±8.8[a]	304±15.9[a]
Amaryl (0.1 mg/kg)	883±14.9[b]	701±11.6[b]	31±0.3[b]	28.9±0.3[b]	527±13.6[b]	866±8.6[b]
LBAE (200 mg/kg)	1035±24.7[a,b]	888±7[a,b]	35.6±0.2[a,b]	34.7±0.4[a,b]	467±10.9[a,b]	681±19.3[a,b]
LBAE (400 mg/kg)	974±12.7[b]	877±3.5[a,b]	34.5±0.3[a,b]	33.7±0.4[a,b]	488±17.4[a,b]	700±13.7[a,b]

Data were expressed as mean±SE (n=8). Statistical analysis was carried out by one-way analysis of variance, $P<0.05$; followed by LSD test for multiple comparisons. LBAE, lemon balm aqueous extract; MDA, malondialdehyde; NO, nitric oxide; TAC, total antioxidant capacity. [a]$P<0.05$, significant from normal control. [b]$P<0.05$, significant from diabetic control.

Figure 1



Effect of lemon balm aqueous extract on the level of cluster of differentiation 4 (pg/g) in liver and kidney of normal and diabetic rats. (a) Significant from normal control at $P<0.05$. (b) Significant from diabetic control at $P<0.05$.

with Amaryl as well as both doses of LBAE reduced TNF-α contents, whereas Amaryl and high-dose of LBAE only elevated CD4 contents when compared with diabetic rats (Figs 1 and 2).

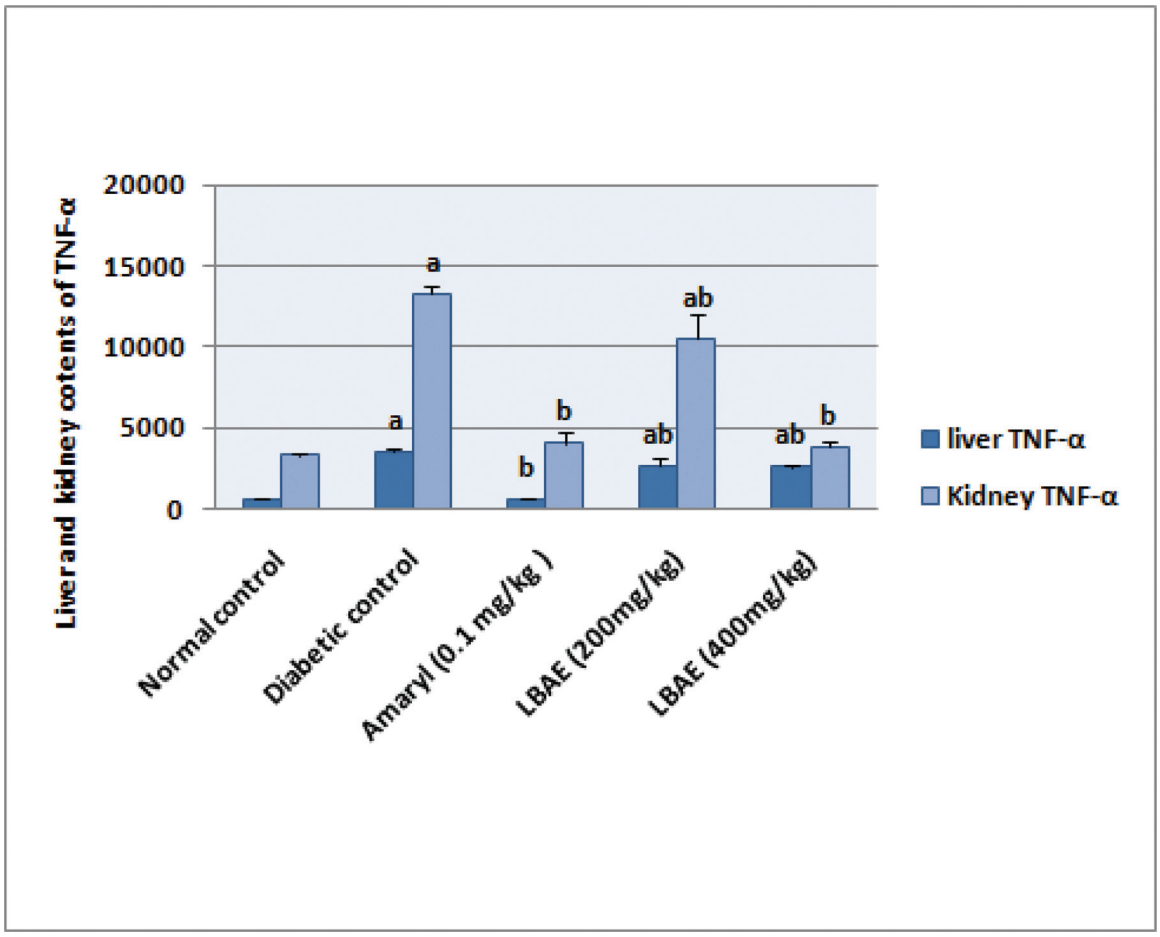
Histopathological results

The light microscopical examination of the pancreatic section from the control rats revealed normal size of β-cells of islets of Langerhans (Fig. 3a). In contrast, pancreatic section from diabetic control group showed small-size, atrophic pancreatic islets of Langerhans (Fig. 3b). Pancreatic section from Amaryl (0.1 mg/kg) group showed pancreatic islets of Langerhans were shaped regularly and arranged evenly (Fig. 3c). Pancreatic section from low-dose (200 mg/kg) and from high-dose (400 mg/kg) treated groups showed that pancreatic islets of Langerhans were shaped regularly and arranged evenly (Fig. 3d and e, respectively).

Discussion

Diabetes mellitus is a syndrome of metabolic disorder of carbohydrate, protein, and fat [19]. Plants that have antidiabetic activity and proven long-term safety should be used in lipid metabolic disorders and cardiovascular diseases [20,21]. The current study was carried out to show the effect of LBAE on STZ diabetic rats.

Figure 2



Effect of lemon balm aqueous extract on the level of tumor necrosis factor-α (pg/g) in liver and kidney of normal and diabetic rats. (a) Significant from normal control at $P<0.05$. (b) Significant from diabetic control at $P<0.05$.

Blood glucose level was increased whereas level of serum insulin was decreased in diabetic rats compared with normal rats. These are in line with Adaramoye *et al.* [22] who used STZ in inducing hyperglycemia in rats. Using STZ is accepted in animal models of diabetes mellitus because it resembles human diabetes mellitus [23] which may eventually leads to renal damage and hepatotoxicity [24].

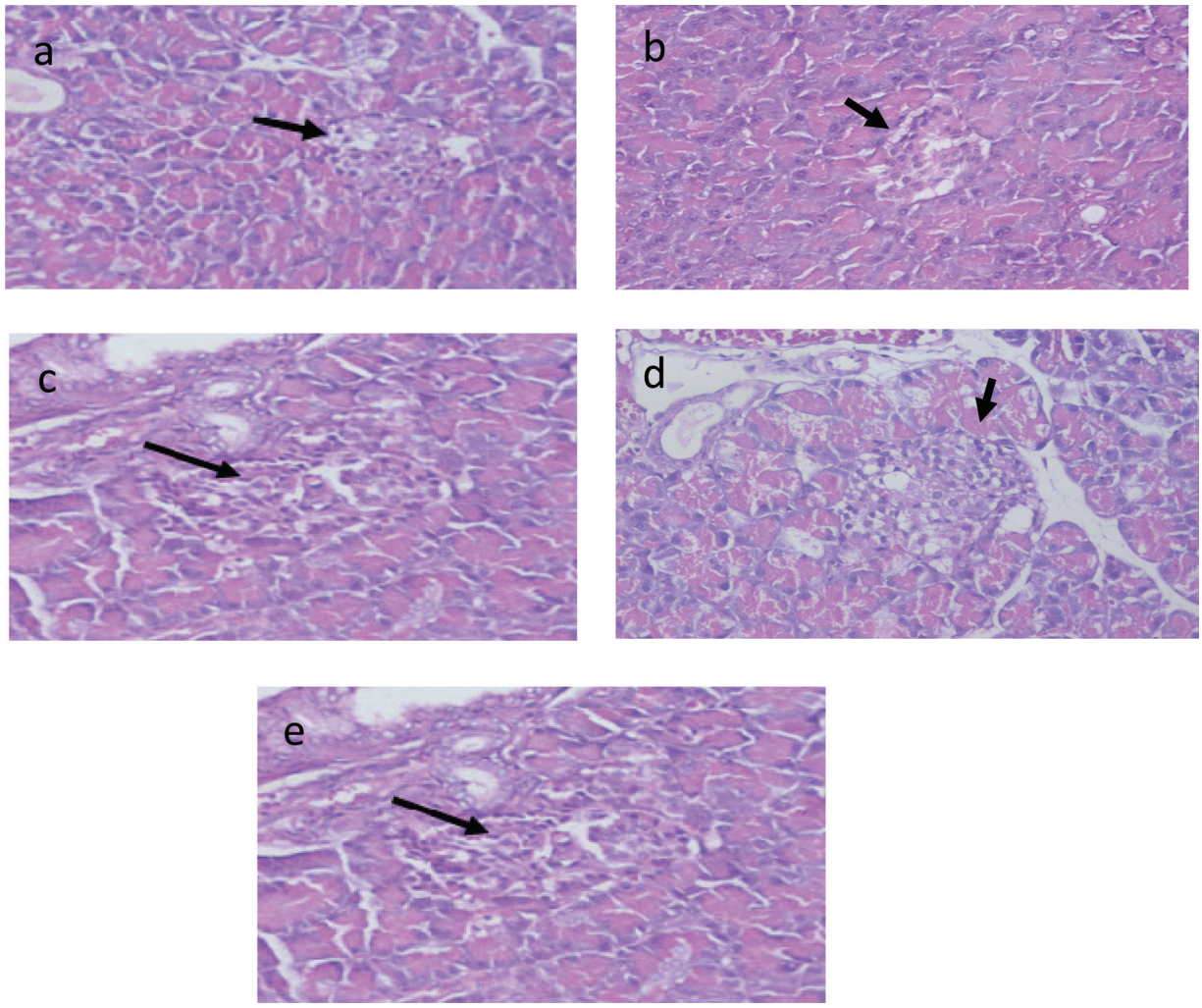
In this study, LBAE caused a reduction and an elevation in levels of blood glucose and serum insulin, respectively, when compared with diabetic rats. The essential oil of *M. officinalis* has antidiabetic effect evidenced by adjusting hepatic gluconeogenesis. The study carried out by Chung *et al.* [21] showed the hypoglycemic effect of plant extract at low doses via regulating glucose uptake as well as suppressing gluconeogenesis [21].

Oxidative stress is the main cause of diabetes complications [25]. These complications may originate from high blood glucose levels, where the production of reactive oxygen species and FRs exceed the capacity of the organism to defend leading to disruption of cellular reduction–oxidation balance [26–28].

The administration of STZ-induced β-cells destruction through NO and inhibition of enzymatic FR scavenging activity in pancreatic tissue [29]. The present study showed that LBAE produced regeneration of the pancreatic β-cells that is evidenced by the elevation of TAC and the reduction of NO level as compared with STZ-induced diabetic group. After damage, pancreatic β-cells replenish the lost cells owing to their proliferating capacity [30]. Our histopathological results showed that the LBAE was responsible for the proliferation of pancreatic β-cells and its normal morphology. Aqueous extract of *M. officinalis* has antioxidant effect owing to its total polyphenolic and flavonoid constituents [31]. Previous study showed the pharmacological effects of volatile terpenoids of *M. officinalis* oils [32].

This study exhibited the hepatoprotective, renoprotective, and pancreatoprotective activities that are highly associated with its antioxidant activity [33,34].

Figure 3



(a) Pancreatic section from normal control group showed normal size of β-cells of islets of Langerhans shown by arrow (H&E, ×400), (b) pancreatic section from diabetic control group showed small-size, atrophic pancreatic islets of Langerhans, which were shown by arrow (H&E, ×400), (c) pancreatic section from Amaryl (0.1 mg/kg) group showed pancreatic islets of Langerhans, which were shaped regularly and arranged evenly shown by arrow (H&E, ×400), (d) pancreatic section from low-dose (200 mg/kg) treated group showed pancreatic islets of Langerhans, which were shaped regularly and arranged evenly shown by arrow (H&E, ×400), (e) Pancreatic section from high-dose (400 mg/kg) treated group showed pancreatic islets of Langerhans, which were shaped regularly and arranged evenly shown by arrow (H&E, ×400).

In diabetic condition, elevated TC and TG levels along with decreased HDL level are confirmed [35]. Administration of STZ in the current study changed lipid profiles through increased levels of TC, TG, LDL, and LDL and reduced HDL level when compared with normal control rats. These findings also happened in high-fat-diet diabetic rats [36].

Daily drinking of M. officinalis tea can regulate TG and cholesterol in humans [29]. In addition, the potential effect of *M. officinalis* may suppress hypercholesterolemia, hyperlipidemia, and lipid peroxidation in the liver of rats [30]. The study of Changizi-Ashtiyani *et al.* [37] on hypercholesterolemic rats has shown that *M. officinalis* reduced serum cholesterol, LDL, and triglyceride. These results may be related to *M. officinalis* antioxidant properties which increase the level of thyroid hormone or the quercetin compounds in the plant, which possess inhibitory effect on lipid peroxidation [38].

Zarei *et al.* [39] found that liver enzymes levels were reduced when receiving *M. officinalis* in hypercholesterolemic group. Accumulation of lipids in the liver stimulates hepatic dysfunction leading to the increases in liver enzymes levels, particularly alanine aminotransferase [40]. On the contrary, increase in lipid levels in liver hyperlipidemia induces the liberation of FRs [41].

The hepatoprotective effect of *M. officinalis* extract may be owing to antioxidant properties of its phenolic compounds that possess FR scavenging capacity

through inhibition of cytochrome system and flavonoids that increase the antioxidant enzymes capacity, protecting the cells against glutathione depletion [21].

In this study, serum levels of creatinine and urea were elevated in the STZ-diabetic rats. These results are consistent with the previous studies of Alderson *et al.* [42] and Adisa *et al.* [43]. Elevated levels of serum urea produced nephrosclerosis, glomerulonephritis, and even tubular necrosis [44]. Our data indicated that LBAE caused reduction in serum levels of creatinine and urea in STZ-diabetic rats, indicating its protective effects against diabetes-induced renal dysfunction [45].

Our STZ diabetic model induced inflammation as it increased liver and kidney contents of TNF-α that endorsed by changes in immune cell function, as it decreased liver and kidney CD4 contents as compared with normal rats. In type 2 diabetes; inflammation is in correlation with T-cell subset imbalance [46,47]. Immunosuppressant effect of STZ may be related to its action on bone marrow and important T cells [48,49]. STZ produced defection of insulin action and β-cell apoptosis leading to immune responses. The proper protein synthesis and normal T-cell functions are related to appropriate uptake of glucose [50]. Zhang *et al.* [51] declared that CD3 and CD4 levels were reduced in patients with diabetes.

In this study, the anti-inflammatory effects of LBAE, which has been shown to decrease liver and kidney TNF-α levels and increase CD4 level as compared with diabetic rats, may be attributed to its bioactive phenolic contents especially flavonoids and rosmarinic acid with their hepatoprotective and anti-inflammatory actions that suppress many enzymes included in the inflammatory activity [52,53].

Conclusion

This study asserts antihyperlipidemic and antidiabetic effects of LBAE in STZ-induced diabetic rats and this will encourage its use as antidiabetic agent. In addition, its beneficial effects on reducing both kidney and liver functions, as well as TNF-α, and enhancing CD4 expression of diabetic rats suggest the possible ameliorating role of the plant extract against secondary complications of diabetes. Further studies are needed to determine active components of LBAE, which have more therapeutic effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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