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Therapeutic effect of *Commiphora molmol* (Myrrh) on hepatic Insulin Receptors and glucose transporter in diabetic rats

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Diabetes mellitus is the causative disease of vascular problems that lead to structural and functional alterations as well as organ malfunction. Aim of the study to clarify the antidiabetic influences of aqueous extract of *Commiphora molmol* (Myrrh), including measurement of hepatic insulin as well as glucose homeostasis in alloxan-induced diabetic rats. Thirty adult female albino rats (120-140 g) were divided into 3 groups: Group I: marked as control, Group II: diabetic rats (injected with 120 mg of Alloxan/kg B.wt) and Group III: treated rats received oral doses of *Commiphora molmol* aqueous extract (0.5g/kg B.wt.) for 30 days. Oral administration of the plant extract considerably lowered the sugar as well as insulin levels towards the normal values of the control group. The rats were sacrificed, blood samples were kept individually for biochemical analysis, and the liver tissue of each animal was removed for gene expression of glucose transporters-4 (GLUT-4), insulin receptor (IR), insulin receptor substrate-1 (IRS-1) and histopathological examination. Result of the present experiment showed the plant ameliorated MDA and TAC levels and improvement of lipid metabolism. Myrrh could be considered as a diet supplement in the procedure of diabetes treatment through insulin signals improvement.

Keywords: Diabetes. *Commiphora molmol*, insulin receptor, glucose transporters, MDA, TAC.

INTRODUCTION

One of the most important metabolic disorders is *Diabetes mellitus* (DM) which is considered as a global disease worldwide. It is characterized by total or relative deficiency of insulin secretion, which leads to persistent hyperglycemia. The illness could be divided into two main categories, type I(T1DM) of which is frequently linked to total-cell apoptosis. While the other type (type II) is linked to the loss of beta cells, impaired insulin production, and insulin resistance. Obesity, becoming older, and other inherited traits are all strongly linked to type 2(T2DM) diabetes (Ansari et al. 2022).

Although the main cause of T2DM is still unclear, several researchers studied that this disorder may be contributed to a number of complicated genetic and environmental variables, including age, sex, aberrant lipid metabolism and obesity. in addition to a familial diabetic history (Sadeghi et al. 2020). Estrogen hormones are known to contribute to a female's life longer than males by

protect them against T2DM and oxidative stress (Díaz, 2019). The mode of action of T2DM is obviously well known. Normally, there is relationship among activity and secretion of insulin. Any impairment in this relationship, abnormal levels of blood glucose are resulted. β-cell dysfunction and Insulin resistance (IR) are standard markers of T2DM. Several researchers have stated that an impaired lipid profile is closely related to IR. IR also may be associated with an elevation of very-low-density lipoprotein (VLDL), triglyceride (TG) and decreased levels of high-density lipoprotein (HDL). So, lipids are considered as risk factors for T2DM (Sadeghi et al. 2020). These risk factors could be also attributed to an incidence of cardiovascular disorders (Ansari et al. 2022). Glucose transporters (GLUT) are carriers that facilitate the entrance of glucose into cell via insulin signaling cascade which is initiated by IR. GLUT-4 is different from other transporters, it acts as insulin-dependent (Hall and Hall, 2020; Yaribeygi et al. 2019). Insulin sensitivity is determined by the number of insulin receptors in the body; therefore, any decrease in IR will have a substantial impact (Mohamed et al. 2019).

As insulin regulates the blood glucose level, Liver also has a vital role in regulation blood sugar as well. Oxidative stress caused by diabetes has tissue damage in patients with diabetes (Niedowicz and Daleke, 2005). Despite the exact mechanism of this damage in diabetes is still of unknown cause, the increase in free radical production is believed to be one of its main destructive mechanisms (Goboza, 2019).

Many synthetic anti-diabetic medications consumed a lot of money or have adverse side effects. On the other, searching for medicinal herbs with hypoglycemic action

are needed to treat diabetics. These herbs can help diabetic patients to avoid these issues (expensive medications and side effect) and are considered of low-price medicines that have little or no adverse effects (Sharma, 2012; Sotoudeh et al. 2019). One of these herbs is *Commiphora molmol* that named as myrrh or Murr in Arabic. It is an aromatic gum resin which is characterized by a high content of bioactive ingredients such as flavonoids, tannins, saponins, polyphenolic compounds, triterpenoids, alkaloids and volatile oils, in addition to furanodienes that exhibit a wide variety of therapeutic uses as well as analgesic activity (Shen et al 2012; Shalaby and Hammouda, 2014; Daradka et al. 2021). Myrrh is ancient medicinal herbs, which was extracted from *Commiphoras* species and originated in the Horn of Africa and southern Arabia. There were few and limited sources of supply for this medicinal plant, which was also a significant source of spices. To transport this valuable commodity over great distances and across numerous nations to the significant foreign markets of Egypt, Mesopotamia, Persia, Greece, and Rome, the incense trade and trade routes were created. Geographical distribution of myrrh (Ben-Yehoshua & Hanuš 2014). Myrrh is used as a carminative, anti-inflammatory, astringent, analgesic, anti-septic, diuretic, emmenagogue, and expectorant among other medicinal purposes. Myrrh has historically been utilized by Unani doctors to treat a set of disorders, including gynecological conditions such cervical stenosis, amenorrhea, menorrhagia, leucorrhea, and pelvic inflammatory disease. Additionally, it is used to treat wounds, ulcers, and a number of gastrointestinal, urinary tract, and respiratory conditions. It also functions as an abortifacient and galactagogue (Fahad and Shameem 2018; Daradka et al. 2020). Recently, limited research has focused on myrrh's effects on the pancreas and its possible antidiabetic capabilities despite its widespread use in conventional medicine.

The influence of *Commiphora myrrh* in the present study on IR, IRS1 and Glut4 gene expression in hepatic cells of mouse was investigated to elucidate the molecular basis of antidiabetic potential of *Myrrh.*

MATERIALS AND METHODS

Plant resins

Identification and collection of plant resins

Oleoresin gum of *Commiphora myrrha* was collected in September 2020 from a wild tree growing in Wadi Noeman, Makkah, Saudi Arabia (21°21'55.98" N 40°11'27.03" E) and kind dedicated by Roushdy M.M. (professor of Microbiology). M. Fadl, Professor of Plant Taxonomy at Taief University, kindly identified the tree. The obtained samples were kept in the Herbarium of the Biology Department at Taif University (voucher specimen ID: Wadi Noeman, 2019, 10512 [TUH] Roushdy M.M.). The quality of oleoresin gum of *Commiphora myrrha* is determined based on its transparency, color, odor, and duration of storage: the gum should not be stored for more than three months and should be transparent, with a golden to brown-yellowish color.

Preparation of *Commiphora molmol* (Myrrh)

Myrrh dried powdered resin (50 g) was rinsed with distilled water and allowed to dry at 60°C overnight. It was then immediately extracted for 48 hours at room temperature using 125 mL of tap water. After extraction, Whatman No. 1 paper was used to filter the myrrha. The filtrate was concentrated in a rotary evaporator with regulated temperature and lowered pressure, and they were then dried at 24 °C room temperature and refrigerated at 4°C.

Experimental animals

A thirty adult female Swiss albino rats of similar age (6 weeks), weighing 120–140 g, purchased from the Egyptian Organization for Biological Products and Vaccines (Helwan, Cairo, Egypt) were used in the present research. All rats were kept in individual conventional cages (3rats per cage) and maintained at an appropriate temperature (28 ± 2°C) and humidity conditions (50% ± 10%) with a standard 12-hour light/dark cycle and ad libitum access to water and basal meal (Reeves et al. 1993) for 7 days as an adaptation period before the onset of the experiment. The diet was manufactured by El Nasr Pharmaceutical Company and purchased from El-Gomhouria Co. Egypt. All rats were weighed, and their body weights recorded each week throughout the feeding period. All protocols for their use in this investigation were approved by ethical council for animal research topic, the National Hepatology & Tropical Medicine Research Institute (NHTMRI) in Egypt, accepted experiment protocol (approval number REC A5-2021).

Induction of Diabetes Mellitus

Diabetes was occurred in overnight fasted animals using a single intravenous injection (120 mg/kg body weight) (Kumar et al. 2011) of monohydrate alloxan dissolved in saline (Sigma Chemicals Co., St. Louis, MN, United States). All of the Alloxan-injected animals developed diabetes, and 30% of them perished throughout the experiment. Serum glucose of the investigated animals was tested using a glucometer 48 hours after injection of alloxan, and those who have sugar levels more than 300 mg/dl were declared diabetic.

Experimental design

The investigated animals were allocated into 3 groups (n = 10 per group).

Group I (GI): they were designated as control group (GI). They were fed a basal diet and supplied with distilled water (1ml/rat body weight), maintained under the same laboratory conditions, and left without neither alloxan induction nor myrrh treatment.

Group II (GII): they were designated as diabetic group, they were fed a basal diet, maintained under the same laboratory conditions and injected with a single intraperitoneal injection of 120 mg Alloxan per kg of body weight (those who have sugar levels more than 300 mg/dl were declared diabetic).

Group III (GIII): they were designated as treated group, they were fed a basal diet, maintained under the same laboratory conditions, injected with Alloxan (120 mg/kg body weight) to induce DM and then treated with Myrrh (0.5g/ Kg b.wt) (Helal et al. 2005) dissolved in distilled water. The Myrrh treatment was administered orally by gastric intubation for 30 days. The rats were weighed weekly.

Collection of blood and tissue samples

After 30 days, the rats were fasted overnight and intraperitoneally anesthetized with urethane (99%, Aldrich) 1 g/kg body weight. Samples of blood were collected from the retro-orbital venous plexus in sterile test tubes. The samples were left to coagulate at 37°C for 15 minutes, centrifuged at 4,000 rpm for 15 minutes until the sera were resulted. The sera were separated, and the samples were then stored at -18°C for further biochemical analyses. The whole liver of the sacrificed animals was collected, dried by using filter paper and weighted, then immersed in phosphate buffer solution PBS (pH 7.4). Liver samples were kept (at -80°C) for biochemical analyses, gene expression determination and for histological examination.

Biochemical analysis

Sera analysis

A series of biochemical tests, including fasting plasma glucose, was measured using a Bio diagnostic kit, Egypt (colorimetric method), serum levels of insulin were assessed using ELISA kit of rat insulin, (Glory science Co., USA), The Homeostasis Model Assessment for assessing β-cell function (HOMA-B) and Insulin Resistance (HOMA-IR) were calculated according to Pickavance et al. (1999) and Heald et al. (2007) respectively, Glycosylated hemoglobin (HbA1c) was measured using the specific kit (Spectrum, Egypt). Total cholesterol, triglycerides and high-density lipoprotein (HDL) were evaluated using Bio-diagnostic kit (Egypt), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) has been calculated according to Mousavi et al. (2012). Serum total antioxidant capacity (TAC) and Malondialdehyde (MDA) were measured according to Miller et al. (1993) and Jiang et al. (2018), respectively.

Prepare of Hepatic Homogenizer for estimation of Lipid

According to Bligh and Dyer (1959), the hepatic total lipid and triglyceride (TG) were extracted, then were estimated using a commercial assay kit (Bio-diagnostic, Egypt Egypt) by enzymatic colorimetric method.

Prepare of Hepatic Homogenizer for estimation of Malondialdehyde (MDA)

According to Nishikimi et al. (1972). Hepatic cell homogenizer was prepared. Approximately 0.5 g of tissue have been mixed with an ice-cold potassium phosphate buffer (0.05 mM) solution (pH 7.3), yielding a five percent (w/v) whole hepatic homogenate. Centrifugation of the homogenates have been at -4°C for 15 minutes at 5000 rpm, and supernatant have been used to assess malondialdehyde levels (MDA).

Quantitative real time polymerase chain reaction (q-RT-PCR)

RNA isolation

Total hepatic RNA has been separated using RNeasy (QIAGEN Mini Kit) with the instructions of the manufacturer. RNA concentration has been estimated by spectrophotometrically using NanoDropND-1000 (Thermo Fisher Scientific, USA). finally, checked the purifications of the isolated RNA.

Reverse transcription

The Reverse Transcription System was utilized to synthesize cDNA from extracted RNA (Promega, Madison, WI, USA). RTase buffer (10), deoxy nucleotide triphosphate (dNTP) mixture (10 mM), MgCl2 (25 mM), oligo d(t) primers (given in Table 1), RNase inhibitor (20 U), and avian myeloblastosis virus (AMV) reverse transcriptase (20 U/L) were incubated at 42°C for 1 hour with total RNA.

Table 1: Primer sequences used to q- real time -PCR

Gene	Sequence of Primer	Accession Number
GLUT4	F: 5´CAAAGCATCGACCAGTGCTA3´	XM_006246596.3
	R: 5´TGGACAGCACTGACTTCCAG3´	
IR	F: 5´CAGCAAGCAGGTCATTGTTTCA3´	NM_017071.2
	R: 5´TGGGTGGGTTTGGGCTCC3´	
IRS1	F: 5´GGACTTGAGCTATGACACGGG3´	NM_012969.1
	R: 5´GCCAATCAGGTTCTTTGTCTGAC3´	
β-actin	F: 5´ATCATCACCTTTGCCGAGTC3´	NM_031144.3
	R: 5´ACAGGTCACTGCCTTCCTTG3´	

Glucose transporter 4 (GLUT4), insulin receptor (IR), insulin receptor substrate 1(IRS1)

Quantitative of real time -PCR (q-RT-PCR)

The ABI PRISM 7500 quick sequence detection system (Applied Biosystems, Carlsbad, CA, USA) was used to perform Q-RT-PCR utilizing gene-specific forward and reverse primers (10 M), SYBR Green Master Mix (Applied Biosystems), cDNA, and nuclease-free water under universal cycling conditions. The relative transcription of

the genes under investigation was calculated using the comparative threshold cycle approach. The data were normalized to -actin, which served as a housekeeping genotype control.

Histological and morphometric examinations

Hepatic sections (three animals from every group) have been fixed in Neutralize formalin buffer (10%). Sections have been embedded paraffin, cut off 4-µm slices then dyed by hematoxylin and eosin **(Teixeira et al. 2000).**

Statistical analysis

The results have been expressed as mean ± SE. The Social Science Statistical Package (SPSS) version 23 (Chicago, USA) was used to carry out one-way analysis of variance (ANOVA), and the post-hoc-test, least significant difference analysis (LSD) to compare the studied groups. At $p < 0.05$, the difference was deemed statistically significant.

RESULTS

The effects of Myrrh on Glucose, insulin, HbA1c, HOMA-IR, HOMA- β

As shown in Table 2., fasting glucose levels were significantly ($P < 0.0001$) elevated (437 mg/dl) in GII, HbA1c and HOMA-IR (8.7%, 0.94 respectively) results were significantly ($P < 0.0001$) elevated in the same group. It is obvious that, the blood sugar, HbA1c as well as HOMA-IR levels were significantly increased by extremely 4.8, 1.7 and 2 folds respectively when compared with results of GI. On the other hand, levels of blood sugar, HbA1c and HOMA-IR in GIII (83 mg/dl 6.01%, 0.32 respectively) were ($P < 0.0001$) decreased to values close to control group.

From results of Table 2, It is also clear that serum insulin as well as HOMA- β% were estimated. The results showed that, serum insulin and HOMA- β% levels were significantly ($P < 0.05$) lowered to 0.87 mU/l with 2.2 folds and 0.86 % with 28 folds for serum insulin and HOMA- β% respectively in Diabetic group. On the other hand, levels of insulin and HOMA- β% in GIII (1.56 mU/l and 33.02 % respectively) were increased to values close to control group.

The effect of myrrh on the serum lipid profile

Results of Table 3 revealed that, HDL-C levels were reduced to approximately 30% in Diabetic group (26.3 mg/dl) in comparison to control group. In treated group, the level was (33.08mg/dl) enhanced to reach the normal levels of control. Serum total cholesterol (TC) as well as Serum triglyceride (TG) values (270 mg/dl and 194 mg/dl respectively) were significantly ($P < 0.001$) increased in group (III) when compared with group(I). In contrast, GIII had lower serum levels for these TC and TG (183 mg/dl and 107 mg/dl respectively) compared to those in the Diabetic group, although they were still higher than those of the control group.

The effects of myrrh on total antioxidant capacity (TAC) and malondialdehyde (MDA) concentrations in serum

Results of Table 4 revealed that, there was a significantly reduction ($P < 0.05$) in serum TAC levels (0.6 mmol/l) as well as a significant elevation ($P < 0.05$) of serum MDA (29.2 nmol/ml) in GII when compared to group(I). Results of GIII showed that the impairment effect that was produced by Alloxan induction was improved in the case of TAC (1.5 mmol/l) and MDA (14.4 nmol/ml) levels to values approximately close to the values of GI.

The effect of myrrh on hepatic total lipid, triglyceride and MDA

Data represented in Table 5 showed that, there was a significant increase of serum total lipids ($P < 0.001$), (98.9 mg/g), triglyceride ($P < 0.002$) (15.85 mg/g) and MDA ($P < 0.001$) (8.3 mg/g) by 2.6, 1.9 and 2.7 folds, respectively compared to GI and GIII groups. The results showed that rats of GIII had more improved levels of total lipids (58.25 mg/g), triglyceride (10.85 mg/g) and MDA (4.3mg/g) than the rats of GII. This result indicates that myrrh showed an improving effect on total lipids even in the presence of alloxan action.

Table 2:The effects of Myrrh on serum glucose, fasting insulin, HbA1c, HOMA-IR index and HOMA- β% as well as total antioxidant capacity (TAC), Malondialdehyde (MDA) concentrations in alloxan-induced diabetic rats after 30 days.

Parameters	Differences/(Unit)	Control group (GI)	Diabetic group (GII)	Treated group (GIII)
Glucose	Value (mg/dl)	92±2.1 [a]	437±16.5 [b]	83±2.6 [ab]
	Change versus control (%)	--	375	-10
	Change versus diabetic (%)	-79	--	-81
Insulin	Value (mU/l)	1.93±0.03 [a]	0.87±0.03 [b]	1.56±0.04 [ab]
	Change versus control (%)	--	-55	-19
	Change versus diabetic (%)	122	--	79
HOMA- IR	Value	0.44±0.01 [a]	0.94±0.02 [b]	0.32±0.01 [ab]
	Change versus control (%)	--	114	-27
	Change versus diabetic (%)	-53	--	-66

HOMA- β	Value (%)	24.89±1.85 [a]	0.86±0.06 [b]	33.02±4.06 [ab]
	Change versus control (%)	--	-97	32
	Change versus diabetic (%)	2795	--	3739
Hb A1c	Value (%)	5.01±0.09 [a]	8.7±0.17 [b]	6.01±0.1 [ab]
	Change versus control (%)	--	74	20
	Change versus diabetic (%)	-42	--	-31

The mean results were expressed as mean ± SE.
a: significance vs Diabetic group, b: significance vs Control group.
The mean difference was significant at $P < 0.05$.
HOMA-IR: homeostasis model assessment of insulin resistance; HbA1c: Glycated hemoglobin; TAC: total antioxidant capacity; MDA: Malondialdehyde concentrations.

Table 3: The effects of Myrrh on serum lipid profile in alloxan-induced diabetic rats after 30 days.

Parameters	Differences/(Unit)	Control group (GI)	Diabetic group (GII)	Treated group (GIII)
TG	Value (mg/dl)	84±2.7 [a]	194±2.88 [b]	107±3.03 [ab]
	Change versus control (%)	--	132%	28%
	Change versus diabetic (%)	-96%	--	-95%
TC	Value (mg/dl)	132±2.69 [a]	270±7.08 [b]	183±4.4 [ab]
	Change versus control (%)	--	105%	39%
	Change versus diabetic (%)	-51%	--	-32%
HDL-C	Value (mg/dl)	37.4±1.11 [a]	26.3±0.92 [b]	33.08±1.0 [ab]
	Change versus control (%)	--	-30%	-12%
	Change versus diabetic (%)	42%	--	26%
LDL-C	Value (mg/dl)	79.4±3.1 [a]	205±7.04 [b]	128.83±4.1 [ab]
	Change versus control (%)	--	158%	62%
	Change versus diabetic (%)	-61%	--	-37%
VLDL-C	Value (mg/dl)	16.72±0.54 [a]	38.74±0.78 [b]	21.47±0.61 [ab]
	Change versus control (%)	--	132%	28%
	Change versus diabetic (%)	-57%	--	-45%

The mean results were expressed as mean ± SE.
a: significance vs Diabetic group, b: significance vs Control group.
The mean difference was significant at $P < 0.05$.
TG: Triglyceride, TC: Total cholesterol, HDL-C: High density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol, VLDL-C: very Low-density lipoprotein-cholesterol.

Table 4: The effects of Myrrh on Total antioxidant capacity (TAC), Malondialdehyde (MDA) concentrations in alloxan-induced diabetic rats after 30 days.

Parameters	Differences/(Unit)	Control group (GI)	Diabetic group (GII)	Treated group (GIII)
TAC	Value (mmol/l)	1.73±0.13[a]	0.6±0.05 [b]	1.5±0.13 [ab]
	Change versus control (%)	--	-65%	-13%
	Change versus diabetic (%)	188%	--	150%
MDA	Value (nmol/ml)	11.3± 0.4 [a]	29.2± 1.1 [b]	14.4± 0.7 [ab]
	Change versus control (%)	--	158%	27%
	Change versus diabetic (%)	-61%	--	-51%

The mean results were expressed as mean ± SE.
a: significance vs Diabetic group, b: significance vs Control group.
The mean difference was significant at $P < 0.05$.
TAC: total antioxidant capacity; MDA: Malondialdehyde concentrations.

Table 5. The effects of Myrrh on the hepatic T lipid, TG and MDA in alloxan-induced diabetic rats after 30 days.

Parameters	Differences/(Unit)	Control group (GI)	Diabetic group (GII)	Treated group (GIII)
T. lipid	Value (mg/g)	38.2±3.5 [a]	98.9±3.3 [b]	58.25±2.6[ab]
	Change versus control (%)	--	159%	53%
	Change versus diabetic (%)	-61%	--	-41%
TG	Value (mg/g)	8.23±0.18 [a]	15.85±0.25 [b]	10.85±0.21[ab]
	Change versus control (%)	--	93%	52%
	Change versus diabetic (%)	-48%	--	-32%
MDA	Value (nmol/mg protein)	3.1±0.2 [a]	8.3±0.3 [b]	4.3±0.3 [ab]
	Change versus control (%)	--	168%	39%
	Change versus diabetic (%)	-63%	--	-48%

The mean results were expressed as mean ± SE.
a: significance vs Diabetic group, b: significance vs Control group.
The mean difference was significant at $P < 0.05$.
T lipid: Total lipids, TG: Triacylglycerol, MDA: Malondialdehyde

Gene expression

At the molecular level, (Fig 1, 2 and 3) revealed that alloxan down-regulated the expression of the IR, IRS-1 and GLUT4 genes in liver ($p < 0.05$), compared with the control group. While treating diabetic animals with the aqueous Myrrh extract was improved the genes expression ($p < 0.05$), compared with the group (II), However, when compared to normal rats, the expression of these genes was dramatically reduced.

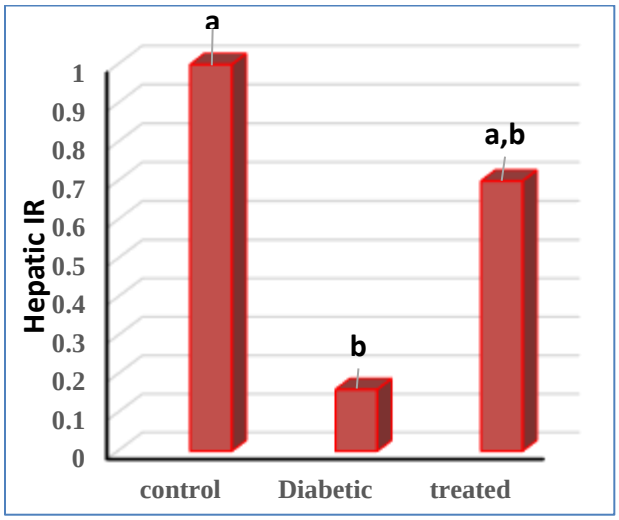

Figure 1: Hepatic IR gene expression of the various groups. Expression was estimated by q-Real Time-PCR and the results were normalized by β-actin. Data were presented as Mean ± SE. Data was presented as Mean SE. ANOVA was used to analyze the data, followed by LSD. At p<0.05, the mean difference is significant. Every group had 10 rats. a: significance vs Diabetic group, b: significance vs Control group.

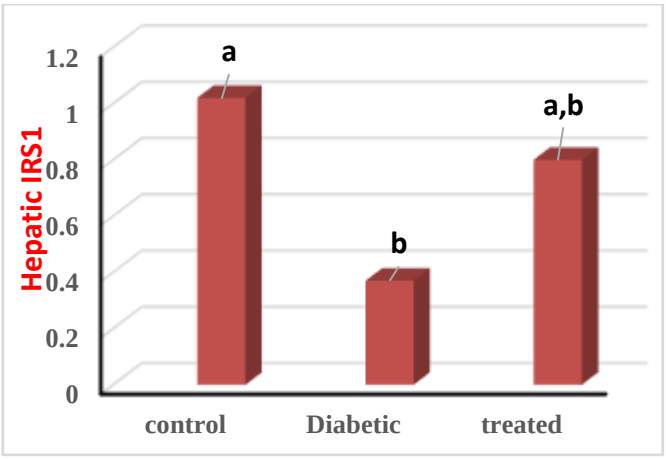

Figure 2: Hepatic IRS1 gene expression of the various groups. Expressions were estimated by q-Real Time-PCR and the results were normalized by β-actin. Data were presented as Mean ± SE. Data was presented as Mean SE. ANOVA was used to analyze the data, followed by LSD. At p<0.05, the mean difference is significant. Every group had 10 rats. a: significance vs Diabetic group, b: significance vs Control group.

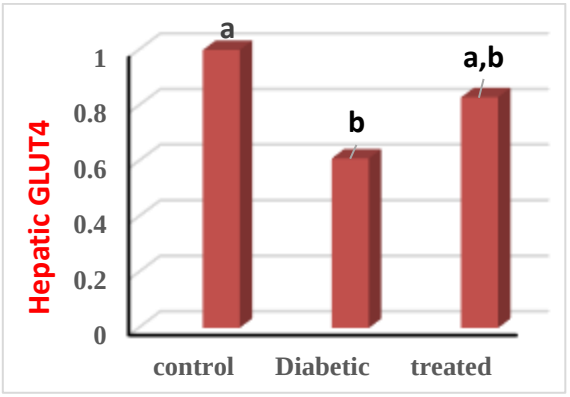

Figure 3: Hepatic GLUT4 gene expression of the various groups. Expression was estimated by q-Real Time-PCR and the results were normalized by β-actin. Data were presented as Mean ± SE. Data was

presented as Mean SE. ANOVA was used to analyze the data, followed by LSD. At p<0.05, the mean difference is significant. Every group had 10 rats. a: significance vs Diabetic group, b: significance vs Control group

Histological analysis

In a trial to confirm the action of the plant extract on the morphology of the tested animal's tissues, histopathological analyses were performed. As shown in (Fig 4C, 4D and 4E), liver cells of GII showed a thickening with collagen and fibroblastic cells proliferation in addition to infiltration of inflammatory cells

in the capsule. The portal area also showed oedema, and inflammatory cells infiltration along with dilatation of the portal vein. Moreover, periductal fibrosis was detected surrounding the bile ducts at the portal area. (Fig 4F) also exhibited that; such pathological changes that were obviously noticed in GIII were greatly improved to be approximately normal in the case of GIII. However, inflammatory cells infiltration has been still observed in the portal area and this may exert the destructive effect of Alloxan. Although this was still seen in such group, but all the other changes were unseen.

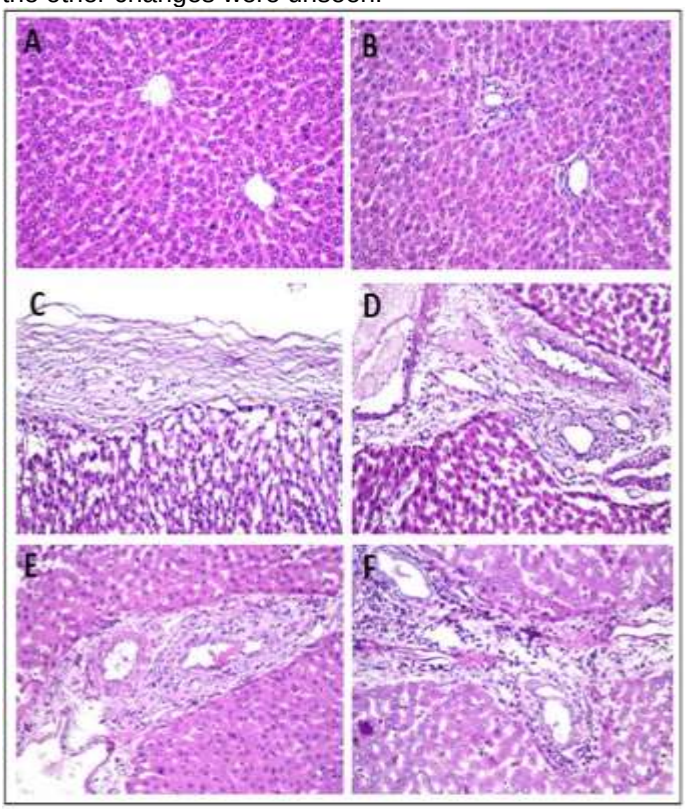

Figure 4: Photomicrographs of hepatic slices from normal (A&B), diabetic (C&D&E), and Myrrh extract-treated rats (GIII) (F). Normal rats had normal histological liver tissue structure (A&B), but diabetic rats had thickening in the capsule with collagen, fibroblastic cells, and inflammatory cells infiltration (Figure 4C). Edema, inflammatory cell infiltration, and portal vein dilatation.

DISCUSSION

The present work is a promising study that was focused on the detection and extraction of useful products from plant origin in a trial to treat some diseases that have more attention worldwide. *Diabetes mellitus* is one of such diseases that causes global issues, and many people suffering from it till now, and in some circumstances, it may lead to death. In the fact that, there are various hypoglycemic medications that are used extensively throughout the world with different mechanisms and of variable efficiencies. Unfortunately, these medications for some reasons may have undesirable effects. Therefore, searching for new substances from natural sources is in demand which give the hope to find some materials devoid of or have little side effects and could be used safely for human being. So, this study was conducted to extract antidiabetic as well as antioxidant ingredients form *Commiphora myrrha* resin and use the Swiss albino rats as a model for this *In Vitro* study. Diabetes was induced by alloxan which is selectively damages insulin-secreting pancreatic β-cells.

Diabetes is a set of metabolic diseases which recognized by increased of blood glucose level and dyslipidemia (Windari et al. 2019). Hyperglycemia could be identified as an elevation of blood sugar due to inability of glucose molecules to diffuse through the cellular bilayer lipid membrane on the bases of their polar nature and big size. However, entrance of glucose is mediated by many structurally related transporters called glucose transporters (GLUTs) (S Song et al. 2021). Matching between insulin and insulin resistance (IR) leads to induction and phosphorylation of IRS-1, that possesses tyrosine kinase activity. Tyrosine phosphorylation drive a series of downstream pathways that stimulates the GLUT translocation to the cell membrane (Eid et al. 2020). Dyslipidemia is characterized by an elevated lipid profile levels (triglycerides, total cholesterol, LDL-C and VLDL-C) due to inhibition of lipolysis and stimulation of hepatic lipogenesis led to defect in insulin secretion resulting in elevation of hepatic triglycerides and cholesterol as well (Schaalan et al. 2009).

Results of the present research clarified that, as a result of alloxan effect, rats of diabetic group (GII) showed an elevation of blood sugar, HbA1c and HOMA-IR with decreased levels of fasting insulin and HOMA- β% to 2.2 folds and 28 folds respectively. Levels of blood sugar, HbA1c and HOMA-IR in GIII were significantly decreased ($P < 0.0001$) to values close to control group (GI) when compared to GII. On the other hand, levels of insulin and HOMA- β% in GIII were increased to values close to control group. This could be explained as that the oral administration of *C. molmol* (Myrrh) at a dose level of 0.5 g/Kg for one month considerably recovered the hyperglycemic effect and the serum insulin level near to values of control. Al-Romaiyan et al. (2021) has been

reported that *C. myrrha* had a direct effect on β-cell by modifying the signaling pathways of stimulation and secretion of insulin rather than increasing insulin synthesis. The present result is also agreed with Iftikhar et al.(2020) and Ajiboye et al. (2021) who stated that, alloxan could induce hyperglycemia accompanied by high level of HbA1c and calculated HOMA-IR as well as affecting the β-cell function as observed from HOMA-β value leading to low serum insulin level.The aqueous extract of *C. molmol* improved the impairment effects (that was induced by Alloxan) in levels of TAC and MDA to values approximately close to the values of GI. Despite, the fact that oxidation is a necessary metabolic activity for living creatures to create energy for many biological activities, oxygen-centered free radicals and certain other reactive oxygen species (ROS) could cause severe and harmful effects to cells and tissues (Ozsoy et al. 2008). The antioxidant influences of *C. molmol* could be attributed to its ingredients like terpenes (specifically sesquiterpene), flavonoids and phenolic compounds (Daradka et al. 2021). In streptozotocin-induced diabetic rats, *Commiphora mukul* greatly reduced lipid peroxidation by preserving activation of the main antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxide) (Bellamkonda et al. 2011).

Dyslipidemia was substantially improved by using the aqueous extract of Myrrh. This improvement could be related to amelioration in insulin sensitivity after taking Myrrh. Apparently hypolipidemic efficacy of Myrrh could well be attributed to the existence of plant sterols (guggulsterone), that have mentioned in both human and animal studies to lower LDL-C and triglyceride levels despite raising blood HDL levels. Guggulsterone has antagonistic action to the receptor of bile acid and inhibitor of pancreatic phospholipase, who regulates the cholesterol and fat absorption in intestine (Yu et al. 2009). Moreover, Singh et al. (1990) illustrated that reduction of serum cholesterol in rats treated with guggulsterone was relevant to improved absorption of LDL through hepatic receptor mediated endocytosis. Orabi et al. (2020) illustrated that *Commiphora myrrha* ethanolic extract improved the hyperglycemia, dyslipidemia and hepatic tissues lipid peroxidation in high fat-fed rats through increasing protein expression of adiponectin and leptin as well as its antioxidant activity.

In the present study, alloxan induced diabetes significantly down-regulates IR, IRS-1 and GLUT4 gene expression in rat hepatocytes. Insulin's primary activity is to control hepatic glucose uptake by GLUT4 (Shan et al. 2011) .It was found that myrrh enhanced insulin receptors in rats and reduced insulin expression through GLUT4, thereby exhibiting anti-diabetic properties (Karnieli and Armoni. 2008).

Interestingly, Myrrh enhanced a decrease in the insulin receptor expression that protect mice from diabetes. Liver is the master organ of insulin regulation since it is the major site for insulin clearance (Peiris et al. 1986). In most studies involving adults, an association between intrahepatic for accumulation and reduced hepatic insulin extraction has been found (Hakim et al. 2019). Additionally, there is a reverse link between insulin clearance and NAFLD (Kotronen et al. 2008). Moreover, hyperglycemia in diabetes induces the overproduction of ROS (Volpe et al. 2018) which impairs hepatic insulin signaling by mitigating recruitment and activation of IRS-1 and IRS-2 and diminishing the subsequent downstream activation of phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT) cascade (Matsuzawa-Nagata et al. 2008; Yang et al. 2014) Antioxidant activities and hypolipidemic of Myrrh may be the major factors that restore the hepatic insulin signaling. Based on the biochemical data, the liver pathology of diabetic rats showed several pathological abnormalities including t hickening of the collagen matrix, fibroblast proliferation, an d inflammation. The pathological lesion in diabetic tissues treated with Myrrh were similar to those shown in untreated diabetic rats, but the severity was slightly lower. These data are similar to those obtained by Hassanzadeh-Taheri et al. (2021)

Further, the effects of commercially available *Commiphora myrrha* on *gene expression* of alloxan - inducing diabetic rats were tested. There was an improvement of gene expression in hepatic tissues due to the presence of terpenes (specifically sesquiterpene), flavonoids and phenolic compounds. Although, the present study employed different useful techniques to evaluate the pharmacological effects of *Commiphora myrrha*, our findings are limited by the lack some information about the precise molecular mechanisms as well as the identity of the compounds involved in the plant responsible for such anti-diabetic effect. As far as I know, this study is the first record to test the effect of myrrh on a gene *expression* to improve diabetic complications.

CONCLUSION

This present study concluded that, the current work showed that, *C. molmol* (Myrrh) extract has considerable antihyperglycemic characteristics that are mediated by a set of processes, including the excitation of insulin secretion and antioxidant activity. The presence of flavonoids, phenolic chemicals, and terpenes may be the cause of its antihyperglycemic and antioxidant benefits. It would be a hope that, *C. molmol* (Myrrh) and its phytoconstituents may be helpful as dietary supplements for the control of diabetes.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors were contributed in this article from the suggestion of article topic to the writing of the final form.

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