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Research Article

Antidiabetic Activities of Methanolic Extracts of *Marrubium vulgare* Leaves in Rats

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Abstract

This study was carried out to evaluate the chronic effect and possible mechanism of action of the methnolic extract (MEex) of *Marrubium vulgare* using normal and streptozotocin-induced diabetic rats, and in vitro methods. Administration of the MEex at a dose of 200 mg/kg daily on diabetic rats for 5 weeks showed a significant lowering ($p < 0.05$) of the blood glucose level, serum urea, uric acid and creatinine and correction of the lipid profiles compared to diabetic rats. However, MEex significantly increased ($p < 0.05$) the glucose uptake by liver and skeletal muscle. The increase in glucose uptake was also shown when the liver and skeletal muscle was treated by 5mg/kg of glibenclamide. Furthermore, the effect of this extract on glucose absorption in the everted rat jejunum showed that MEex at concentrations of 2 mg/mL and 5 mg/mL significantly reduced the glucose absorption of the jejunum ($p < 0.05$). Similarly, the absorption of glucose was also inhibited by 1 mg/mL of acarbose ($p < 0.01$). These results suggest that the effect of MEex may be due to extrapancreatic mechanisms. This antidiabetic activity is, at least partly, due to modulation of glycogen synthesis and inhibition of intestinal glucose absorption.

1. INTRODUCTION

The antiquity of use of plants by human beings for the treatment of various disorders and health care is not known. The herbal and natural products of folk medicine have been used by men since the advent of human race[1]. Recently, scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these time tested remedies. Folk medicine in different cultures has a long history of ancestors creating primitive medicines during their struggles against natural calamity and disease. While searching for food, the ancient humans found that some foods had specific properties of relieving or eliminating certain diseases, and maintaining good health. It was the beginning of herbal medicine, which has been playing an important role in the health care[2].

Marrubium vulgare L. (Lamiaceae), commonly known as "Horehound", is a widespread Mediterranean plant used in folk medicine to cure a variety of diseases, and is widely distributed in Morocco[3]. The leaves and young flowering stems are used as antiseptic, antispasmodic, antidiabetic, diuretic, strongly expectorant and tonic[4]. Many of the activities traditionally ascribed to *Marrubium vulgare* were confirmed by intensive modern research and clinical trials, such as antioxidant[5], analgesic[6], anti-inflammatory[7], and anti-oedematogenic[8], furthermore, extracts of this plant have shown some effects on type–II diabetes[9] and, very recently, on hepatoprotective activity[10].

The aim of this study was to investigate the blood glucose lowering potential of *Marrubium vulgare* at dose 200 mg/kg and to investigate the possible mode of action using rat model.

2. MATERIELS AND METHODS

2.1 Plant materiel

The leaves of *Marrubium vulgare* were collected in August in a region of Meknes, Morocco. Botanical investigation was carried out after herborization of the collected material and a voucher specimen was deposited at the Herbarium (Laboratory of Biochimical and Pharmacognosy, Faculty of Sciences Meknes).

2.2 Preparation of the extracts

The leaves were dried and finely powdered. The powder (200g) was extracted by maceration with dichloromethane (1L), ethanol (1L) and methanol (1L). The extracts were concentrated under reduced pressure and pooled, yielding 2.17%, 10.54% and 15.11% (w/w) of dichloromethane extract (DCex), ethanol extract (ETex) and methanol extract (MEex) respectively. The extracts were dissolved in distilled water. The residues obtained were stored in airtight bottles in a refrigerator for further use.

2.3 Animal material

Wistar rats (weight: 180-210g, age: 6-8 weeks) used in the present study were obtained from the Animal house of the Department of Biology, University Moulay Ismail of Meknes. The animals were kept and maintained under laboratory conditions of temperature (23 ± 2 °C), humidity (65 ± 10%) and light (12-h light/dark cycles). They were also allowed free access to food and water *ad libitum*. Experimental procedures were also examined and approved by internal ethical committee for animal welfare.

2.4 Induction of experimental diabetes

Normoglycemic Wistar rats. A freshly prepared solution of Streptozotocin (STZ) (45mg/kg body weight) in 0.1M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1mL/kg body weight to overnight fasted rats. After 72 hours of STZ administration, development of diabetes was confirmed by glucose analysis of tail vein blood. Rats with blood glucose levels more than 200 mg/dL were selected for the treatment[11].

2.5 Evaluation of the hypoglycemic effect of the organic extracts in healthy rats

To investigate hypoglycemic effect the normal fasted rats (16 h) were allotted into eight groups (n=6). Extracts and control substances were administered by gavage.

Groups 1 served as controls, receiving 1 mL of distilled water (DW). Groups 2 and 3 received 100 and 200 mg/kg body weight of the DCex.

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Groups 4 and 5 received 100 and 200 mg/kg body weight of the ETex.
Groups 6 and 7 received 100 and 200 mg/kg body weight of the MEex.
Groups 8 received glibenclamide 5 mg/kg body weight.
Blood samples were collected from tails 2, 4 and 6 h post-treatment with DW or treatments for the determination of serum glucose levels.

2.6 Evaluation of the hypoglycemic activity produced by the daily administration of MEex to STZ-diabetic rats
Wistar rats were randomly divided into four groups (n = 6), and each group was treated with:
Group 1: Normal control rats were administrated 1mL of (DW).
Group 2: STZ-diabetic control rats were administrated 1mL of (DW).
Groups 3: STZ-diabetic rats were administrated MEex (200 mg/kg body weight)
Group 4: STZ-diabetic rats were administrated glibenclamide (5 mg/kg body weight)
All extracts were administered orally during 5 weeks. Blood samples were collected from tails on a week 1, 2, 3, 4, and 5 and centrifuged at 2500 rpm for 15 min at room temperature. Next, plasma was obtained for analytical determination of various biochemical parameters including blood glucose level, serum urea, uric acid and creatinine levels as well as the lipid profile. Additionally the body weight was monitored weekly during the experimental period.

2.7 Effect on liver and skeletal muscle glycogen content
Eighteen STZ-diabetic rats were assigned randomly into three equal groups (n = 6). One group was orally administrated with 200 mg/kg of MEex, second group treated with glibenclamide at dose of 5 mg/kg and the other group with 1mL of DW daily for 5 weeks. On day 1 post-treatment, these rats were sacrificed after ether anesthesia and portions of their livers and skeletal muscles were removed and blotted free of blood. Glycogen content was determined using a spectrophotometric method as described in detail by Borst et al[12].

2.8 Investigation of the activity of glucose absorption from the intestine
Wistar rats were fasted for 16 hours before the experiment. Animals were sacrificed after ether anesthesia, and dissected to remove the small intestine[13]. Specifically, the jejunums were removed shortly after death and were placed in a Krebs' bicarbonate solution (which contains the composition of: 120 NaCl, 4.5 KCl, 1 $MgSO_4$ 1.8 Na_2HPO_4, 0.2 NaH_2PO_4, 1.25 $CaCl_2$ and 25 $NaHCO_3$); all are measured in mmol quantity and the other end was also tied. The wet sacs were immersed in a flask containing 40 mL of Kreb's solution and incubated in a shaker bath (37 °C and 30 rpm) and gassed with 95% O_2 and 5% CO_2. The jejunums were everted by using a glass rod and tied at one end with a ligature thread. A Krebs' bicarbonate solution with 140 mg/dL glucose was injected into the everted jejunums, and the other ends were tied into jejunal sacs, 1.5 cm long. The jejunal sacs (n=6) were incubated in 10 mL of a Krebs' bicarbonate solution with the addition of the plant extract, following this protocol.
Group 1: incubated in a Krebs' bicarbonate solution with glucose, 140 mg/dL (control group).
Groups 2-4: incubated in a Krebs' bicarbonate solution with glucose, 140 mg/dL and MEex at 1, 2 and 5 mg/mL.
Group 5: incubated in a Krebs' bicarbonate solution with glucose, 140 mg/dL and 1 mg/mL of glibenclamide.
Group 6: incubated in a Krebs' bicarbonate solution with glucose, 140 mg/dL and 1 mg/mL of acarbose.
After the incubation (60 min), the sacs were dissected, and the solution in each sac was examined for its glucose level[14, 15].

2.9 Statistical analysis
Statistical analysis involved use of the Statistical Package for Social Sciences (SPSS) version 20. Data are expressed as the mean ± S.E.M., and statistics were performed using one-way analysis of variance (ANOVA). Significant differences between the control and treatments groups were determined using Dunnett's test and $P < 0.05$ was considered significant.

3. RESULTS
3.1 Hypoglycemic effect of the organic extracts in healthy rats
The effects of the organic extracts with *Marrubium vulgare* and glibenclamide on the serum glucose concentration in normal fasted rats are depicted in Table 1.
Basal glycemia did not change significantly in the control groups, whereas glibenclamide reduced it at 2, 4 and 6 h ($P < 0.05$) in normal rats. The MEex presented a significant hypoglycemic effect at doses of 200 mg/kg after 2 and 4 h, inducing an important hypoglycemia. The DCex and ETex at dose of 100 and 200 mg/kg respectively, did not significantly change of the blood glucose level.

Table 1: Hypoglycemic activity of organic extracts of *Marrubium vulgare* on healthy rats

Group	In fasting	2 h	4 h	6 h
Control	73,69±1.33	74,69±2.10	62.01±5.38	66.12±4.65
DCex 100	71,13±2.14	65,22±1.98	63.55±6.41	76.71±5.33
DCex 200	84,31±1.47	76,51±4.67	69.38±3.13	70.36±3.04
ETex 100	90,79±3.01	79,80±5.36	76.41±4.94	88.91±2.51
Etex 200	72,12±3.56	66,00±4.88	76.01±5.12	71.64±2.11
MEex 100	71,33±1.48	62,27±6.01	66.01±4.19	60.92±5.33
MEex 200	87,82±3.35	67,02±2.85*	66.91±3.04*	69.21±4.19
Glibenclamide	71,17±2.03	53,13±3.60*	56.01±1.04**	63.25±4.34*

*The values are mean ± SEM, n= 6, when compared with healthy control, * = p<0.05, ** = p<0.01.*

3.2 Hypoglycemic activity produced by the daily administration of MEex
Table 2 and 3 shows the changes in body weight and biochemical parameters after the chronic administration of MEex to STZ-diabetic rats.
Body weight of STZ- diabetic rats was found to be significantly ($p < 0.05$) less compared to normal rats (Table 2). After three weeks of treatment with MEex, the body weight had significantly ($p < 0.05$) increased compared to diabetic control. Progress in weight gain of animals in drug-treated group was continued to be observed till the end of the study. Body weight of the animals in glibenclamide treated group also increased significantly ($p<0.01$) on a week 2 (Table 2).
The treatment with MEex at a dose of 200 mg/kg for five weeks exhibited a significant ($p < 0.05$) decrease in the fasting blood glucose in STZ-induced diabetic rats as compared to diabetic control (Table 2). Blood glucose level of diabetic animals started decreasing from the third week of drug treatment that was continued to maintain till fifth week, which was comparable to glibenclamide 5 mg/kg. There were a 52 and 60 % decrease in blood glucose level with MEex at a dose of 200 mg/kg and glibenclamide 5 mg/kg treatment, respectively.
The results showed (Table 2) that serum urea, uric acid and creatinine increased, when compared with normal rats. The administration of the MEex (200 mg/kg) significantly decreased serum urea (46%, $p < 0.05$), uric acid (41%, $p < 0.05$) and creatinine (67%, $p < 0.01$), when compared with control diabetic rats.

Table 2: Body weight, glycaemia, urea, uric acid and creatinine levels in plasma after orally daily administration of DW (control), glibenclamide, and a methanol extract of *Marrubium vulgare* to STZ diabetic rats

Group	Week	BW (g)	Blood glucose (mg/mL)	Uric acid (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Group 1	1	199.14±9.35	85.12±3.37	4.12±1.54	55.14±6.87	0.41±0.15
	2	230.25±4.08	70.41±5.34	4.87±2.08	47.98±7.14	0.42±0.08
	3	280.27±19.16	79.88±6.11	5.01±1.37	44.45±5.35	0.39±0.12
	4	270.36±10.48	88.24±2.23	4.05±1.71	34.78±9.11	0.47±0.11
	5	350.18±9.05	64.56±8.15	3.84±0.94	40.16±4.75	0.39±0.14
Group 2	1	200.55±10.13	313.14±10.23[a]	6.45±2.52	70.54±8.08[a]	0.64±0.28[a]
	2	180.24±8.58[a]	315.22±9.33[a]	6.14±2.41	78.46±9.43[a]	0.79±0.41[a]
	3	174.56±10.08[a]	362.34±18.25[a]	5.35±2.91[a]	88.17±6.55[a]	0.99±0.37[a]
	4	181.75±6.14[a]	386.07±9.44[a]	7.01±3.11[a]	73.81±7.75[a]	0.81±0.57[a]
	5	154.54±7.11[a]	401.54±15.01[a]	6.91±2.28[a]	81.85±5.40[a]	1.24±0.49[a]
Group 3	1	186.07±9.31	280.16±19.05[a]	5.87±2.15	65.17±5.01[a]	0.59±0.24*
	2	250.66±11.75	274.84±10.48[a]	4.54±1.55	50.31±7.79	0.46±0.18*
	3	294.75±5.43*	251.58±11.05[a*]	3.47±1.82*	53.10±4.41	0.33±0.17*
	4	280.54±11.01*	190.67±7.76[a]*	4.14±2.83*	41.84±1.95*	0.34±0.10*
	5	279.75±8.44*	191.80±14.33[a]*	3.03±1.05*	44.38±7.19*	0.41±0.11**
Group 4	1	190.84±10.12	200.38±11.22[a]*	4.27±1.14	58.34±6.25*	0.39±0.18*
	2	239.61±9.05**	201.88±11.44[a]*	2.17±1.95[a]*	41.15±3.75*	0.42±0.22*
	3	274.54±8.08*	188.57±13.21[a]*	3.10±0.87*	37.08±9.13**	0.38±0.12*
	4	280.45±12.16**	160.74±19.05[a]*	4.58±1.01	40.57±5.04*	0.40±0.21*
	5	302.11±5.04**	161.53±18.61[a]*	3.88±1.08	42.89±8.18*	0.44±0.19*

*Values are expressed as mean ± SEM, n = 6 in each group. [a] p < 0.05 compared to control, *p < 0.05, **p < 0.01 compared to diabetic control.*

The lipid profiles in control and experimental rats are depicted in Table 3 in STZ induced diabetic rats. The serum triglycerides (TG), total cholesterol (CHO), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels were significantly higher in the STZ diabetic rats compared to those in normal rats, while the high density lipoproteins (HDL) levels were significantly decreased in the STZ induced diabetic rats compared to those in normal rats. Also, the treatment with MEex showed a significant reduction in the CHO, TG, LDL and VLDL.

The treatment with MEex could also significantly increase the HDL levels in diabetic treated rats compared to those in STZ induced diabetic rats and was similar effective in comparison with glibenclamide. The continuous treatment with the MEex of *Marrubium vulgare* brought down the above lipid profiles in the diabetic rats to almost normal levels.

Table 3: Total cholesterol, triglycerides, HDL, LDL and VLDL levels in plasma after orally daily administration of DW (control), glibenclamide, and a methanol extract of *Marrubium vulgare* to STZ diabetic rats

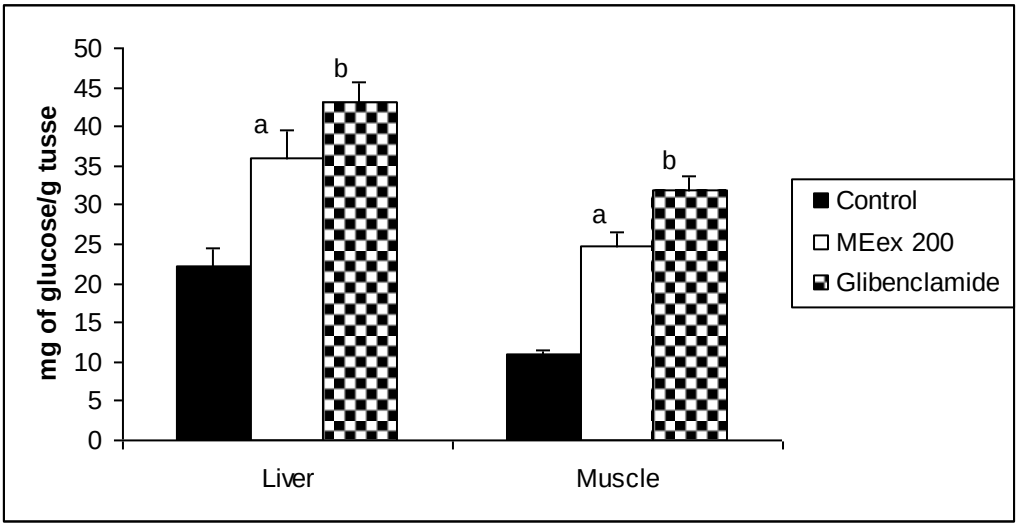
Group	Week	TG (mg/dL)	CHO (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VHDL (mg/dL)
Group 1	1	80.87±2.33	84.18±3.33	30.17±1.05	44.12±2.05	18.49±2.65
	2	96.18±4.11	85.12±2.14	32.10±0.38	42.17±1.34	19.54±1.18
	3	86.47±3.24	87.94±2.47	34.21±1.35	44.64±2.35	18.05±1.55
	4	88.84±2.28	83.51±1.08	33.62±1.18	40.58±1.68	19.03±1.22
	5	90.17±3.01	81.48±2.37	33.31±2.33	43.81±2.52	18.14±2.37
Group 2	1	220.14±5.81[a]	133.45±4.01[a]	28.39±1.04[a]	87.11±3.70[a]	37.21±2.08[a]
	2	214.01±3.56[a]	127.31±5.54[a]	27.65±1.22[a]	80.17±2.22[a]	40.51±3.43[a]
	3	218.54±1.99[a]	138.47±3.88[a]	27.01±2.83[a]	90.24±3.31[a]	42.17±2.55[a]
	4	221.56±4.35[a]	131.28±2.10[a]	28.42±1.22[a]	88.40±1.53[a]	41.88±2.33[a]
	5	219.16±2.44[a]	136.40±4.33[a]	28.57±0.88[a]	98.02±3.44[a]	49.36±3.46[a]
Group 3	1	99.12±3.18*	99.14±3.26*	33.12±1.11*	67.25±1.45*	22.11±1.99*
	2	88.35±2.81*	91.87±1.64*	30.14±1.31	60.28±3.22*	22.21±1.08*
	3	80.57±4.33*	88.11±3.55*	31.58±1.05*	63.57±2.33*	20.20±1.97*
	4	79.10±1.66*	87.34±2.02*	28.51±2.84	51.53±3.08**	19.55±2.38*
	5	81.30±0.95*	79.47±3.15*	29.33±2.12	54.21±2.51*	18.01±2.34*
Group 4	1	84.34±1.68*	71.38±2.66*	33.24±2.08*	41.24±3.11**	20.17±1.58*
	2	76.37±2.50*	80.46±2.37*	30.97±2.02	44.32±1.34**	18.53±3.15*
	3	77.58±1.39*	66.94±3.95**	35.74±1.77*	40.66±2.65**	19.32±1.01*
	4	66.64±2.11**	75.61±3.18*	32.32±1.65*	49.37±1.09**	18.74±1.91*
	5	66.01±1.37**	77.84±2.21*	33.35±0.55*	50.14±1.91**	17.59±2.44*

*Values are expressed as mean ± SEM, n = 6 in each group. [a] (p < 0.05) compared to control, *(p < 0.05), **(p < 0.01) compared to diabetic control.*

3.3 Effect of liver and skeletal muscle glycogen content

Treatment with the extract improved the muscle and hepatic glycogen content by 33 and 28%, respectively in comparison to diabetic group. (Fig 1)

Fig.2. Effect of MEex of *Marrubium vulgare* on liver and muscle glycogen contents in STZ induced diabetic rats. Data are expressed as mean ± S.E.M; n =6, a and b indicates the significant level of differences in hepatic and muscle glycogen contents as compared to control (STZ induced diabetic), respectively ($p < 0.05$).



3.4 The effects of the MEex on glucose absorption in the everted rat jejunum

Table 4 shows that the everted small intestine absorbed glucose during incubation in Krebs' bicarbonate solution over a 60 minute period. The addition of glibenclamide at concentration of 1 mg/mL did not significantly change the amount of glucose absorbed. Moreover, the addition of acarbose at dose of 1 mg/mL significantly ($p < 0.05$) inhibited the absorption of glucose in the everted small intestine when compared to Krebs' bicarbonate solution alone. The amount of glucose absorption of the everted small intestine was also significantly ($p < 0.05$) inhibited by the MEex at a dose of 5 mg/mL (Table 4).

Table 4: Effects of methanol extract of *Marrubium vulgare* on rat intestinal glucose absorption

Groups	Glucose concentration inside the sacs (mg/dL)	% inhibition
Group 1	321.38 ± 14.31	0.00
Group 2	318.17 ± 11.08	0.99
Group 3	301.51 ± 15.16	6.18
Group 4	203.32 ± 20.67*	36.37
Group 5	281.03 ± 19.54	12.55
Group 6	139.77 ± 10.88 **	56.50

Values are glucose concentration inside the sacs after 60 minutes of incubation with or without the extract that expressed as means ± SEM.
*Percentage of inhibitions was calculated based on control group representing 100% of glucose absorption. * P <0.05 and **P<0.01 compared with control.*

4. DISCUSSION

Diverse medicinal plants have been used for the management of diabetes mellitus in various traditional systems of medicine worldwide as they are a great source of biological constituents and many of them are known to be effective against diabetes[16]. Antihyperglycemic effects of these plants are attributed to their capacity to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Numerous plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having antidiabetic effect[17, 18].
Marrubium vulgare has been used in the traditional medicine of various regions of Morocco to treat diabetes[19, 20]. Due to that reason, we decided to evaluate the antidabetic and hypoglycemic effects of some organic extracts on healthy and STZ-induced diabetic rats.
A significant blood glucose reduction was observed in healthy rats after the oral administration of MEex of *Marrubium vulgare* (200 mg/Kg). MEex showed the best hypoglycemic effect on healthy rats and it was time dependent. Moreover, the other organic extracts (Etex and DCex) had slight hypoglycemic effects on healthy rats. On the other hand, glibenclamide caused significantly more hypoglycemia in comparison with the MEex. Therefore, the results demonstrated that MEex of *Marrubium vulgare* induced significant decrease of plasma glucose levels in STZ induced diabetic rats for long term (repeated) administration this hypoglycemic activity was more pronounced in STZ induced diabetic rats. Furthermore, a marked normalization of blood glucose levels was achieved after 3 weeks of oral treatment indicating that the hypoglycemic effect of MEex of *Marrubium vulgare* is cumulative. It seems that MEex prevents the increase in blood glucose levels in diabetic rats without inducing hypoglycemic state. The hypoglycemic effect observed in normal rats suggests that MEex possesses an important pharmacological effect because, in spite of counter regulatory factors, a hypoglycemic activity was observed. This may reveal that the mechanism of *Marrubium vulgare* on glycemic control may be similar to that of glibenclamide, a prototype sulfonylureas, which was supported by previous works[21, 22].
A significant reduction in the body weight was observed in the STZ-induced diabetic rats. The decrease in the weight in diabetes is due to continuous excretion of glucose and decrease in peripheral uptake of glucose and gluconeogenesis which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins[23, 24]. STZ-induced diabetic rats treated with MEex showed an increase in body weight as compared to diabetic control rats, which may be due to its protective effect in controlling muscle wasting *i.e.* reversal of gluconeogenesis.
Diabetes is also associated with hyperlipidemia[25]. The administration of MEex significantly decreased serum triglycerides and cholesterol in diabetic rats. These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin[26]. This extract supplementation also results the significant attenuation in the level of LDL and HDL in serum toward the control level which again strengthen the hypolipidemic effect of this extract.
The diabetic hyperglycemia induces elevation of the serum levels of urea, creatinine and uric acid which are considered as significant markers of renal dysfunction[27, 28], reported that in severe diabetic condition there is an elevated excretion of urea whose concentration can be higher than the normal value. Our data showed that uric acid levels were increased in diabetic rats. This may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation and increased triglycerides and cholesterol[29, 30]. In present study, significant increase in serum urea, uric acid and creatinine levels were

observed in STZ induced diabetic rats compared to normal control rats wich indicates impaired renal function in STZ induced diabetic rats. The treatement with MEex of lowered the above parameters significantly compared to diabetic control rats it showed protective effect of *Marrubium vulgare* on the kidneys.

Glycogen provides an additional source of glucose besides that produced via gluconeogenesis. Because glycogen contains so many glucoses, it acts like a battery backup for the body, providing a quick source of glucose when needed and providing a place to store excess glucose when glucose concentrations in the blood rise. Insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since STZ selectively damages β-cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin influx of glucose[31]. Moreover, this alteration in muscle and hepatic glycogen was normalized by insulin treatment[32]. The ability of MEex to increase liver and skeletal muscle glycogen may be due to activation of glycogensynthase. MEex thus possess antihyperglycemic activity partly due to its ability to increase glycogenesis and increase peripheral up take of glucose.

The effects of natural products on and metabolism in peripheral tissues have also been studied using fragments of the rat small intestine[33]. Among these methods, the isolated rat everted rat jejunum methods were chosen in the present study to evaluate the glucose absorption, in an attempt to elucidate the mechanisms of action of the antihyperglycemic of MEex of *Marrubium vulgare*. It is well known that small intestine possesses an energy-dependent transport process system by means of which glucose and certain other sugar substances can be absorbed against the concentration gradient.

Acarbose, a clinically available alpha-glucosidase inhibitor, has shown its inhibitory action by reducing the digestion of oligosaccharides in the proximal half of the small intestine by prolonging the absorption of monosaccharides after a meal. An acarbose concentration of 1 mg/mL produced the greatest reduction in the intestinal absorption of glucose[34, 35]. In the present study, acarbose significantly reduced glucose absorption at the concentration of 1 mg/mL. However the results from present study showed that MEex of *Marrubium vulgare* inhibited the transport of actively transported sugar. The concentration of glucose accumulated in the rat intestine increased significantly in the presence of MEex of *Marrubium vulgare*. Therefore the MEex of *Marrubium vulgare* produced reductions in glucose absorption. Since previous studies have indicated that flavonoids and terpenoids produce antidiabetic activity[36, 37, 38], these compounds could be responsible for the antihyperglycemic effect of *Marrubium vulgare*, whether acting separately or synergistically.

5. CONCLUSION

The methanolic extract of *Marrubium vulgare* is effective in controlling the elevated blood glucose levels in STZ induced diabetic rats. This antidiabetic activity is, at least partly, due to modulation of glycogen synthesis and inhibition of intestinal glucose absorption.

6. ACKNOWLEDGEMENTS

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