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Research Article

Phytochemical and Antihyperglycemic Studies on Citrus medica l. Leaves (Etrog) Growing in Egypt

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ABSTRACT

From 70% aqueous methanol extract of the defatted powdered leaves of Citrus medica L. var. Etrog, six compounds, namely, β-sitosterol-glucoside (1), sakuranetin (2), 7-O-methylaromadendrin (3), dihydrokaempferide (4), hesperitin (5) and rutin (6) were isolated and identified by physicochemical and spectral data (UV, MS and NMR). Compounds 2-4 are newly reported from the genus and 5 is newly reported from the species. The extract was safe up to 2g/kg.bwt. The histopathological changes on liver, kidney and pancreas were assessed. The antioxidant activity was calculated to 102.9µg/ml. The antihyperglycemic activity exerted a significant reduction in blood glucose level to (105.2±8.35) in diabetic rats after one month of treatment with a dose of 200 mg/kg and to (87.4±6.30) with 400mg, when compared to Gliclazide standard (110.8±7.24) ($P< 0.05$). As a conclusion, the methanol extract of the defatted powdered leaves of Etrog exhibits a significant antihyperglycemic activity which might be attributed to the presence of flavonoid compounds.

Keywords: Citrus medica L., Etrog, flavonoids, antioxidant, antihyperglycemic, histopathology.

INTRODUCTION

Citrus medica L. (Citron, Etrog, itranj in arabic), native to Southeast Asia about 4000 years ago, has been cultivated since ancient times. In antiquity, the etrog was called the Persian or Median Apple. It was used as a symbol of resistance.1 The essential oil of C. medica L. leaves was analysed by GC/MS, HPLC and GC.2 Its antioxidant activity3 and fungitoxic effects4 were studied. The prominent anthelmintic activity against earthworms was reported by (Manoj et al., 2011)5. The tested antimicrobial activity of leaves was lower than of other organs.6 The hypoglycemic activity of Diamante – Citron variety and the insulin secretagogue bioactivity of Finger – Citron was also reported.7 The authors tested previously the estrogenic activity of the leaves.8 As long as the available current literature is concerned the antihyperglycemic or antioxidant activities of the leaves of Etrog (plant under study) are not previously reported.

MATERIALS AND METHODS

Phytochemical study: General Experimental Procedures: UV spectra were recorded on 6800 UV/Vis spectrophotometer JENWAY; EIMS on Jeol JMS – AX 500 Mass spectrometer, NMR on Jeol – ECA instrument at 300 MHz for 1H and 75 MHz for 13C NMR, in DMSO-d6 or CD3OD using TMS as internal standard. TLC was run on precoated Si gel plates using EtOAc/ formic acid/ acetic acid/ H2O (100:10:10:10) as developing system; AlCl3 and vanillin – H2SO4 as spray reagents. Sephadex LH-20 was used for CC (Amersham Pharmacia Biotech B, Uppsala, Sweden) and silica gel 60 for CC (E. Merck, Darmstadt, Germany).

Plant material: The leaves of C. medica L. Etrog (Fig.1), were collected from El Qualyobia Governorate, Egypt, on April, 2009. Plant authentication was performed by Mrs. Therese Labib, Senior Botanist, El Orman Garden, Egypt. A voucher specimen (BUPD20) is deposited at Pharmacognosy Department, Beni-Suef University.

Preparation of MeOH extract: Fresh leaves (2 kg) were air dried in shadow and pulverized to powder (500g). The powdered leaves were defatted with petroleum ether (the latter was tested by the authors in a previous communication.8 250g of the defatted powder were extracted with 70% MeOH, by maceration, on cold, till exhaustion. The collected extracts were concentrated under reduced pressure to give a semisolid mass (36 g).

Isolation of compounds from 70% MeOH extract: Seven grams of the 70% MeOH extract were fractionated over silica gel CC (60X3cm), using n-hexane; n-hexane/ CH2Cl2; CH2Cl2; CH2Cl2/EtOAc; EtOAc; EtOAc/MeOH and MeOH in a way of increasing polarity to give 12 collected fractions according to their behavior on TLC. Fraction 2 (500 mg) was subjected to Sephadex LH-20 CC and gave compound 1. Fraction 5 (1.3g) was resubjected to Si gel CC (55X2.5cm) using n-hexane; n-hexane/ CH2Cl2; CH2Cl2; CH2Cl2/MeOH and MeOH for elution from which subfraction 10 (650 mg) was purified using Sephadex LH-20 CC and MeOH as eluent, to give 8

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Table 1: Effect of 70% MeOH extractive of Citrus medica L. (Etrog) leaves on hemoglobin (Hb), red blood cell count (RBCs) and total leukocytic count (TLC) in *mice.*

Groups	Dose (g/kg) p.o.	Hb (g/dl)	RBCs (10^6/mm^3)	TLC (10^3/mm^3)
Control	Saline	11.2± 0.14	3.8±0.03	5.1±0.29
MeOH extractive	2	9.6± 0.15 @	3.4±0.02 @	3.9±0.10 @

Values represent the mean ± S.E. of five mice for each group.@ P< 0.05: Statistically significant from control (Independent sample t-test).

(a) (b)

Fig. 1: *Citrus medica L. (Etrog) leaves (a) and fruits (b)*

collected fractions. From the collected subfraction 3 (60mg) compounds 2, 3 and 4 were isolated after purification on a Sephadex LH-20 CC using MeOH/H2O (7:3) as eluent. From the collected subfractions 4 (20mg) and 5 (25mg) compounds 5 and 6 were obtained respectively.

Biological Study: Experimental Animals: Mature Albino mice (20 – 25g) were used for acute toxicity study and mature Wister albino female rats (135-150 g) for the antihyperglycemic activity. All the animals obtained from the animal house of NRC were kept under the same hygienic conditions and fed with well balanced normal diet and water ad libitum.

Drugs and kits: Alloxan was purchased from Sigma-Aldrich chemical company, USA; Gliclazide (Diamicron®), Servier Laboratories, Egypt, was purchased from a local pharmacy. Ascorbic acid standard was purchased from Merck, Germany Co., Egypt. Glucosekits (Biodiagnostic, Egypt) were used for the enzymatic determination of glucose adopting glucose – oxidase method. Estimation of lipid profile was performed using Cholesterol (Biodiagnostic, Egypt), Triglycerides (Biodiagnostic, Egypt), HDL-Cholesterol (Biodiagnostic, Egypt) and total lipids (Biodiagnostic, Egypt).

Acute toxicity study: Acute oral toxicity study of the 70% methanol extract of the defatted leaves of C. medica L. (Etrog) was determined in albino mice according to Lorke (1983).9 Animals were observed for 24 hours for any signs of toxicity or death. Two weeks later, blood samples from the retro-orbital plexus of all mice were obtained, estimation of blood hemoglobin (Hb), red blood cell count (RBCs) and total leukocytic count (TLC) were performed. Animals were sacrificed; liver and kidney organs were collected and kept in 10% formalin solution for histopathological investigations.

DPPH Antioxidant activity: The DPPH assay was performed according to the method adapted from Amic et al., 200310 and Phang et al., 2011.11 The absorbance of the reaction mixtures was measured at 520 nm. Methanol was used as blank and DPPH solution was used as control without addition of extract. Ascorbic acid was used as a positive control.

Anti-hyperglycemic assay: Thirty five rats were divided into 5 groups (7 rats each). All rats, except group 1 which was kept as normal non-treated rats (negative control), were rendered diabetic after a single intraperitoneal injection of Alloxan monohydrate in a dose of 135 mg/kg b.wt. Diabetic groups (2-5) were treated as follows: group 2 served as non-treated diabetic (positive control), groups 3 and 4 were orally treated with 70% MeOH extract of the plant at doses of 200 and 400 mg/kg b.wt., respectively, for one month, and group 5 was kept as standard using Gliclazide (Diamicron®) as a reference drug (20 mg/kg b.wt). Blood samples were collected from the venous plexus. Serum was separated and glucose levels were estimated by enzymatic glucose oxidase-method. Cholesterol, triglycerides, low-density lipoprotein (LDL) and, high-density lipoprotein (HDL) were estimated at the beginning, after induction of diabetes and at the end of the experiment.

Histopathological study: The Pancreas of each group was isolated for histopathological examination. After washing in Phosphate buffered saline (PBS) solution, the isolated pancreas was stored in 10% formalin. Paraffin sections of pancreas tissue were stained in haematoxylin and eosin for evaluation of β- cells of islets in light microscope.12

STATISTICAL ANALYSIS

The results were analysed using one way analysis of variance (ANOVA) with Dunett's comparison test, p-values <0.05 were considered statistically significant. Statistical analysis of results, was carried out using analytical software named SPSS statistics 17.0, release (Aug. 23, 2008), Chicago, USA.

Ethical consideration: All the experimental procedures utilized were performed in accordance to the Research Ethics Committee of National Research Centre, Cairo, Egypt under strict compliance of Committee for the purpose of control and supervision of experiments on animal's guidelines for the experimental studies.

RESULTS AND DISCUSSION

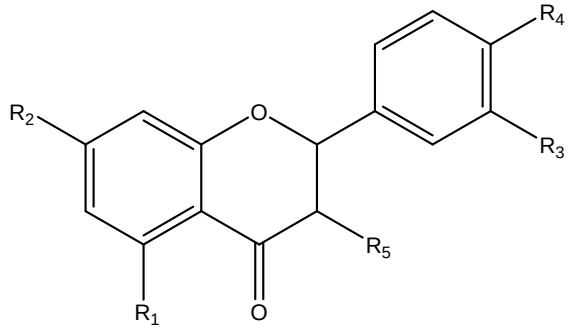
Phytochemical study of the isolated compounds: Compounds 1 β-sitosterol-glucoside (60mg), 5 hesperitin (20mg) and rutin 6 (25mg) were identified by comparison of their spectral data (UV, MS, NMR) with data reported in the literature 13-15 and by comparing their chromatographic behavior and mixed melting points with the authentic samples. Compound 5 which was identified

Table 2. Effect of 70% MeOH extract of Citrus medica L. (Etrog) leaves on blood glucose level in rats.

Groups	Dose / p.o.	Glucose (mg/dl)		
		Basal	After 72 h	After 1 month
Control	saline	93.1±4.62	109.0±3.70 [bc]	112.5±3.62 [b]
Diabetic control	saline	98.7±5.63	246.4±10.56 [a]	172.3±2.09 [ac]
70% MeOH extract	200 mg/kg/day	106.8±5.87	247.0±12.78 [a]	105.2±8.35 [bc]
	400 mg/kg/day	109.3±5.04	240.5±7.83 [a]	87.4±6.30 [abc]
Diamicron ®	20 mg/kg/day	101.8±4.23	237.8±8.49 [a]	110.8±7.24 [b]

Values represent the mean ± S.E. of seven rats for each group.
[a] P< 0.05: Statistically significant from normal control (Dunnett's test).
[b] P< 0.05: Statistically significant from diabetic control (Dunnett's test).
[c] P< 0.05: Statistically significant from diamicron® (Dunnett's test).

Fig. 2. Structures of isolated compounds of *Citrus medica* L. var. Etrog leaves.



	Compound no.	R_1	R_2	R_3	R_4	R_5
Sakuranetin	2	OH	OCH_3	H	OH	H
7-*O*-Methyl-aromadendrin	3	OH	OCH_3	H	OH	OH
Dihydrokaem-pferide	4	OH	OH	H	OCH_3	OH
Hesperitin	5	OH	OH	OH	OCH_3	H

as hesperitin, was previously isolated together with its glycosidic form from genus Citrus 13 and isolated for the first time from this species. The structures of the isolated compounds are presented in Fig. 2.

Compound 2: Sakuranetin: 14mg, yellow amorphous powder, dark purple in UV light, bright yellow with ammonia vapor, Rf = 0.72 (EtOAc/ formic acid/ acetic acid/ H2O (100:10:10:10). UV (MeOH): λmax (log ε) 258, 355 nm, AlCl3 275, 305 sh, 340, 428, AlCl3/HCl 270 sh 305, 340, 400, NaOAc/H3BO3 260, 305 sh, 340, 375; EIMS m/z: 286 (M+, C16H14O5), 193(5%), 167(30%), 119(13%); 1H NMR: (DMSO-d6), δ 12.4 (1H, s, OH), δ 11.4 (1H, s, OH), δ 9.9 (1H, s, OH), δ 9.4 (1H, s, OH), δ 7.4 (2H, d, J = 8.4 Hz,H-2'and H-6'), δ 6.9 (2H, d, J = 8.4 Hz, H-3' and H-5'), δ 6.4 (1H, d, J = 1.5 Hz, H-6), δ 6.2 (1H, d, J = 1.5 Hz, H-8), δ 5.3 (1H, dd, J = 1.5 and 1,5 Hz, H-2), 3.8 (3H, s, OMe-7), δ 3.3 (1H, dd, J = 1.5 and 9.6 Hz , H-3b), δ 3.1 (1H, dd, J = 1.5 and 9.6 Hz, H-3a). These data were in agreement with the previously reported data.16-18 13C NMR (in CD3OD) spectral data revealed the presence of 14 signals which are in agreement with the reported data.17 Compound 2 is proved to be a flavanone by absence of a double bond between C2 – C3 in the C ring, also by the constant presence of 5 – OH , 7- OCH3 substitution pattern at the A ring and a single 4' – hydroxyl group at ring B.19 This compound is identified as Sakuranetin (5,4'-dihydroxy-7-methoxyflavanone). It is isolated for the first time from family Rutaceae.

Compound 3: 7-O-Methylaromadendrin: 13mg, yellow powder, dark purple in UV light, bright yellow with ammonia vapor, Rf = 0.74 (EtOAc/ formic acid/ acetic acid/ H2O (100:10:10:10). UV (MeOH): λmax (log ε) 265, 351nm; EIMS m/z: 302 (M+, C16H14O6), 209 (5%), 167 (30%), 119 (15%); 1H NMR: (MeOD), δ 12.4 (1H, s, OH), δ 9.9 (1H, s, OH), δ 9.4 (1H, s, OH), δ 7.4 (2H, d, J = 7 Hz, H-2' and H-6'), δ 6.9 (2H, d, J = 7 Hz, H-3' and H-5'), δ 6.4 (1H, d, J = 2 Hz, H-8), δ 6.2 (1H, d, J = 2 Hz, H-6), δ 5.2 (1H, d, J = 11 Hz, H-2), δ 4.5 (1H, d, J = 11 Hz, H-3), δ 3.2 (3H, s, OMe-7), δ 2.5 (1H, s, OH). 13C NMR (in CD3OD) spectral data revealed the presence of 14 signals which are in agreement with the reported data.17 UV spectral analysis of compound 3 in MeOH (265, 351nm) and after addition of the different shift reagents suggested a dihydroflavonol skeleton with free hydroxyl groups at positions: 3, 5 and 3' or 4' this was confirmed by 1H NMR which showed the presence of two threo – coupled doublets between 4.5 and 5.0 ppm indicative of a dihydroflavonol structure.20 By comparison with the

Fig. 3: Histopathological changes in liver and kidney

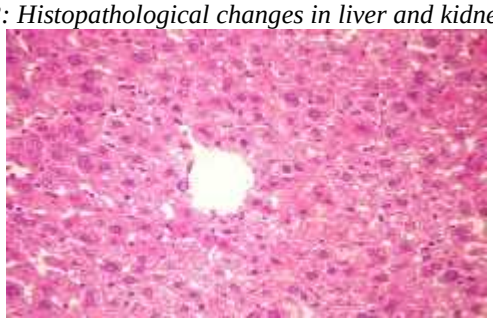


Fig. 3a: Liver of mice from control group showing normal histological structure of hepatic lobule (H&E×400).

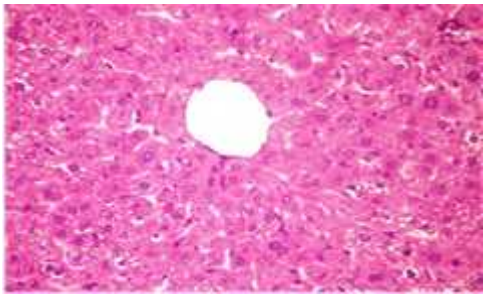


Fig. 3b: Liver of mice from MeOH extract group showing no histological changes (H&E×400).

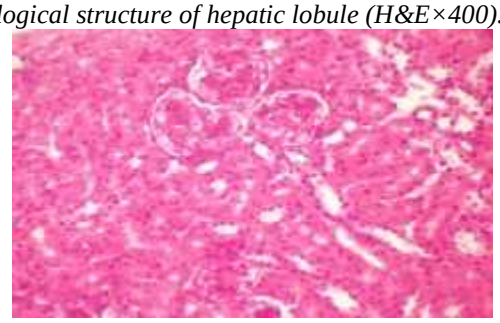


Fig. 3c: Kidney of mice from control group showing no histopathological changes (H&E×400)

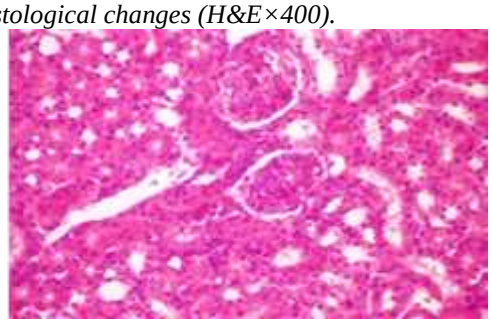


Fig. 3d: Kidney of mice from MeOH extract group showing no histopathological changes (H&E×400).

previously mentioned data.16-18,21 Compound 3 was identified as 7-O-Methylaromadendrin. It is isolated for the first time from family Rutaceae.

Compound 4: dihydrokampferide: 10mg, yellow powder, dark purple in UV light, bright yellow with ammonia vapor, Rf = 0.68 (EtOAc/ formic acid/ acetic acid/ H2O (100:10:10:10). UV (MeOH): λmax (log ε) 265, 351nm. EIMS m/z: 302 (M+, C16H14O6). 1H NMR: (CD3 OD) δ 12.5 (1H, s, OH), δ 9.6 (1H, s, OH), δ 9.1 (1H, s, OH), δ 7.5 (2H, d, J = 8.4 Hz, H-2', H-6'), δ 6.3 (1H, d, J = 8.4 Hz, H-3'), δ 6.1 (2H, d, J = 9.6 Hz, H-5'), δ 5.3 (2H, m, H-6 and H-8), δ 5.2 (1H, d, H-2), δ 5.1 (1H, d, H-3), δ 3.3 (3H, s, OMe C-4'), δ 2.4 (1H, s, OH). From the previously mentioned data and by comparison with the reported data22, compound 4 could be identified as dihydrokampferide and isolated for the first time from the genus Citrus.

Biological Study

Acute toxicity study: Oral administration of 2g/kg.b.wt of 70% MeOH of Citrus medica L. extract showed a significant decrease in hemoglobin (Hb), red blood cell count (RBCs) and total leukocytic count (TLC), Table 1. These results could be attributed to the presence of rutin compound.23

Histopathological changes in liver and kidney: No histopathological changes were observed in liver and kidney after administration of 2g/kg.b.wt which proved the safety of the extract on both organs (Fig.3).

Antioxidant activity of the MeOH extract of Citrus medica L. leaves: 70% MeOH extract of C. medica L. of the defatted leaves showed a significant antioxidant activity (EC50 102.9 µg/ml) when compared to ascorbic acid (EC50 49.28 µg/ml).

Antihyperglycemic Activity: After one month of treatment at doses of 200 and 400mg/kg.b.wt with the 70% MeOH extract of the defatted powder, results showed that there was a significant and a dose dependant decrease in serum level of glucose (105.2±8.35) with 200mg and (87.4±6.30) with 400mg treated doses when compared to the standard Gliclazide (110.8±7.24) and the diabetic control groups (172.3±82.09) mg/dl, (Table 2). Experimental induction of diabetes in rats showed a significant increase in serum glucose, LDL and triglycerides levels together with a significant decrease in serum HDL and no effect on Cholesterol level as compared to normal rats (Table 3). The results of the effect on serum lipids of diabetic rats were recorded (Table 3). The treated dose at 400 mg/kg normalized the elevated serum triglycerides after 1 month of administration. The obtained results were supported by the histopathological findings in pancreas tissue (Fig. 4).

Histopathological study on Pancreas: Results of histopathological study showed β – cells atrophy on administration of 70% MeOH extract at a dose of 200 mg/kg b.wt. (Fig. 4). The administration of 400mg/kg b.wt dose showed no gross lesions in pancreas (Fig. 4d) while the degenerative changes were obvious in diabetic rats group (Fig. 4b). Similar progressive regenerative changes were observed on treatment with the standard Gliclazide (Fig. 4e). The non-diabetic rats, kept as control, showed no histopathological changes (Fig.4a).

The 70% MeOH extract of the defatted leaves of C. medica L. when tested proved to be rich in flavonoids and it was

Table 3: Effect of 70% MeOH extract of *Citrus medica* L. (Etrog) on level of total serum lipids in rats.

Group		Dose/p.o.	Basal	After 72h	After 1 month
HDL (mg/dl)	Control	Saline	65.1±2.54	60.4±1.20 [bc]	56.9±1.33 [b]
	Diabetic	Saline	63.5±3.51	44.9±0.86 [a]	41.8±1.78 [a]
	70% MeOH extract	200 mg/kg/day	71.5±2.69	43.6±0.98 [a]	42.0±1.28 [a]
		400 mg/kg/day	70.5±3.32	43.6±2.34 [a]	50.6±0.93
	Diamicron®	20 mg/kg/day	71.3±1.98	45.3±1.92 [a]	51.7±5.41
LDL (mg/dl)	Control	Saline	85.3±6.07	87.4±4.67 [bc]	82.8±3.81 [b]
	Diabetic	Saline	86.2±3.51	102.7±2.45 [a]	101.4±3.72 [a]
	70% MeOH extract	200 mg/kg/day	88.4±6.93	102.5±4.25 [a]	101.3±8.00 [a]
		400 mg/kg/day	89.5±5.37	103.3±3.97 [a]	89.6±1.98
	Diamicron®	20 mg/kg/day	89.7±3.60	102.8±3.26 [a]	91.1±5.44
Choles-terol	Control	Saline	169.7±6.44	166.9±5.44	156.3±4.17
	Diabetic	Saline	169.4±2.24	186.1±7.58	170.5±5.11
	70% MeOH extract	200 mg/kg/day	183.3±4.49	183.6±7.14	160.1±6.60
		400 mg/kg/day	182.4±2.13	186.4±4.10	169.5±7.21
	Diamicron®	20 mg/kg/day	180.6±3.80	186.7±5.72	159.2±6.60
TG (mg/dl)	Control	Saline	94.4±4.36	98.3±3.86 [bc]	102.3±4.94 [c]
	Diabetic	Saline	98.4±4.33	171.5±6.06 [a]	109.3±3.05 [c]
	70% MeOH extract	200 mg/kg/day	102.6±1.14	164.6±5.17 [a]	117.9±4.96 [c]
		400 mg/kg/day	95.0±4.65	162.0±8.24 [a]	88.3±4.94 [b]
	Diamicron®	20 mg/kg/day	95.3±4.63	175.6±4.37 [a]	81.9±3.73 [ab]

Values represent the mean ± S.E. of seven rats for each group.

[a] *P< 0.05: Statistically significant from normal control (Dunnett's test).*

[b] *P< 0.05: Statistically significant from diabetic control (Dunnett's test).*

[c] *P< 0.05: Statistically significant from Diamicron® (Dunnett's test).*

safe up to 2g/kg p.o., but a significant decrease in hemoglobin (Hb), red blood cell count (RBCs) and total leukocytic count (TLC) in mice [9.6g/dl, 3.4 (106/mm3) and 3.9 (103/mm3)] when compared to the control [11.62g/dl, 3.8 (106/mm3) and 5.1 (103/mm3)] respectively (Table 1). So we have used the fifth and the tenth of the maximum soluble dose in the toxicity study to escape this side effect. Diabetes was induced chemically using alloxan, which is a β-cell toxic compound. It produces oxygen radicals in the body, which cause pancreatic injury through destruction of β-cells of the islets of Langerhans; leading to massive reduction in insulin release and increased blood sugar in animals.24 An abnormality in the lipid profile is considered as a common complication in Diabetes mellitus. Phenolic compounds are reported to be antioxidant25, Vit C, E and carotenoids are known also by their great antioxidant activity.26 Flavanones and flavanols were reported to act as biological antioxidant in cell cultures2 and offer some protection against the early stage of diabetes.27 They also normalize the blood glucose altering the glucose regulatory enzymes.27 They decrease glucose levels and improve glycolytic and gluconeogenetic enzymes in tissues.23 In the Anti-hyperglycemic assay, results showed a significant decrease in glucose level after one month of treatment at the two doses 200 and 400mg (105.2 and 87.4 mg/dl respectively) when compared to the non – treated and the diabetic control groups (112.5±3.62 and 172.3±2.09 mg/dl respectively), (Table 3 & Fig. 2). The tested extract succeeded to completely restore normal glucose level and it significantly prevented further elevation of blood sugar. Therefore, it seems that administration of this extract of C. medica can also inhibit progression and deterioration of hyperglycemia. Antihyperglycemic effect of plants is achieved by enhancing insulin secretion from beta cells, increasing glucose uptake by tissues, decreasing glucose absorption from intestine, inhibiting glucose production in liver, increasing pancreatic tissue regeneration and/or presence of insulin-like agents in plants. Hesperidin and rutin were previously isolated from the peel of C. medica cultivated in Bangladesh .2 Rutin exhibits antihyperglycaemic and antioxidant activities.23 Its Oral administration to diabetic rats decreases fasting plasma glucose, glycosylated haemoglobin and increased insulin, C-peptide, haemoglobin and protein levels. Choi and Kim, 200827 reported that hesperetin can act as a biological antioxidant in a cell culture system representative of a diabetic state and protect osteoblasts from oxidative stress-induced toxicity, which may promote bone recovery in diabetic bone diseases. In general, flavonoids could ameliorate hyperglycemia by protein tyrosine phosphatase 1B (PTP1B) expression in liver.29 7-O-methylaromadendrin reported that it may act by stimulation of glucose uptake into peripheral tissues which is an important mechanism for the removal of glucose in blood and for the management of diabetes mellitus.30 In the present study, treatment with MeOH extract of C. medica significantly decreased the hyperlipidemia, (Table 2). Therefore, the extract most probably can prevent dyslipidemia-related complications of diabetic patients.

CONCLUSION

Fig. 4: Effect of 70% MeOH extract of C. medica L. (Etrog) on pancreas study in mice

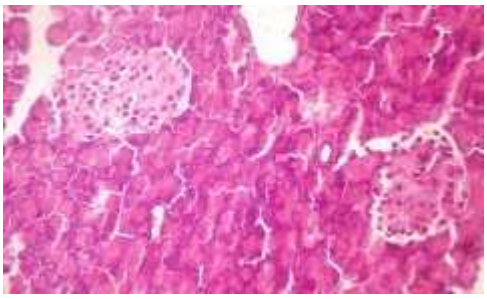


Fig. 4a: Pancreas of rat from control group showing no histopathological changes (H&E X 400).

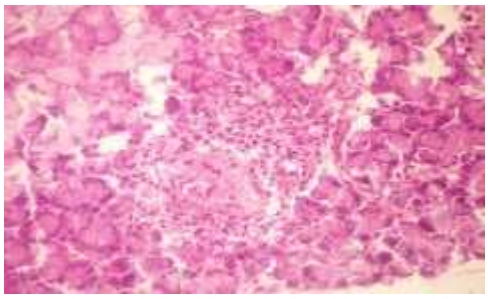


Fig. 4b: Pancreas of rat from diabetic control group showing necrosis of β- cells of islets of Langerhan's (H&E X400).

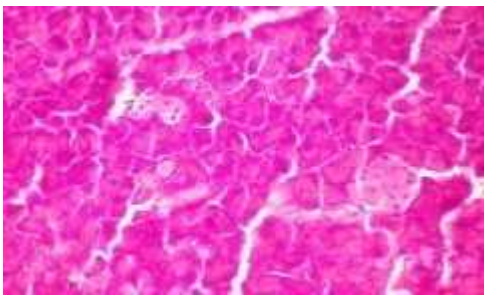


Fig. 4c: Pancreas of rat from (MeOH extract, 200mg/kg) group showing atrophy of β-cells of islets of Langerhan's (H&E X400).

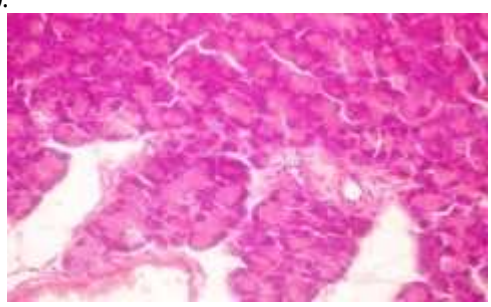


Fig. 4d: Pancreas of rat from (MeOH extract, 400mg/kg) group showing no histopathological changes (H&E X400).

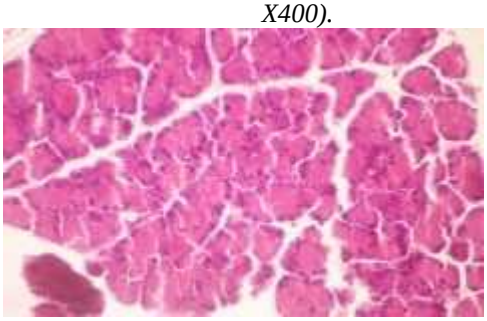


Fig. 4e: Pancreas of rat from Diamicron® group showing no histopathological changes (H&E X400).

The present study demonstrated that the 70% aqueous MeOH extract of the leaves of the defatted powder of C. medica L. var Etrog, has antihyperglycemic effect mainly through its hypolipidemic and antioxidant effects as well as through inhibition of progression and deterioration of glycemia, probably due to the presence of flavonoid compounds. Therefore, it has the potential to be used as a new natural product for the management of diabetes.

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