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Phytochemical and Pharmacological Investigations on *Moringa peregrina* (Forssk) Fiori

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Abstract – Investigation of *M. peregrina* aerial parts revealed the isolation and identification of 4-flavonoidal compounds, quercetin, quercetin-3-*O*-rutinoside (rutin), chrysoeriol-7-*O*-rhamnoside and 6,8,3′,5′-tetramethoxy apigenin. The compounds were identified by TLC, PC, MS, and H^1-NMR. The fatty acids and unsaponifiable matter were studied. The LD_{50} for *M. peregrina* was 113.4 mg/100 g b.wt. Repeated intraperitoneal injection of 1/20 and 1/10 LD_{50} (5.67 mg and 11.34 mg/100 g b.wt.) of defatted alcoholic extract of *M. peregrina* for 30 days induced significant decrease in serum glucose, liver enzymes and lipid components. *M. peregrina* administered i.p., 30 min prior to carrageenan at the above doses significantly inhibited the rat paw oedema response. In acute pain models, namely, the acetic acid-induced writhing and hot-plate assay, *M. peregrina* exhibited marked analgesic properties. In addition, *M. peregrina* administered at time of indomethacin injection inhibited the development of gastric lesions in rats.

Keywords – *Moringa peregrina*, Moringaceae; flavonoids, anti-diabetic, anti-oxidant, anti-inflammatory, analgesic

Introduction

Moringa peregrina (Forssk.) Fiori (Moringaceae) (syn.: *Hyperanthera peregrina* Forssk; *M. aptera* Gaertner, Fruct. Sem.) is known in Arabic as *Habb El Yasar* and *Habb el ban*. The seeds are known as *Habba Ghalia* (Batanouny *et al.*, 1999). Ethnobotanical studies indicate that *M. peregrina* is used to treat headache, fever, abdominal pains, constipation, burns, back and muscle pains, and during labour in childbirth. The plant has been used by the Egyptians since Old and Middle Kingdoms (3000-2000 B.C) (Miller and Morris, 1989). The leaves and the roots, when mixed with water, are used to treat malaria, hypertension, stomach disorders, to expel a retained placenta, and to treat other health problems such as asthma and diabetes (Mekonnen, *et al.*, 1999).

The young seeds of *M. peregrina* are eaten like peas and the mature seeds are fried or roasted like groundnuts. The physical and chemical functional properties of seed protein and lipids of *M. peregrina* were studied (Al-Kahtani and Abou-Arab, 1993). However, no detailed reports were found about the chemical or biological nature of this plant. So the present work was designed to study the phytochemical constituents of the aerial parts of *M. peregrina*. With some pharmacological studies.

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Experimental

Plant material – The aerial parts of *M. peregrina* were collected from southern Sinai during May and June 2002. The plant was kindly identified by Prof. Dr. K. H. Batanouny, Professor of Botany, Faculty of Science, Cairo University to whom the authors are deeply indebted. A voucher specimen was kept in the herbarium of the National Research Centre No.2-6-2002, *Moringa peregrina* (Moringaceae).

Apparatus and Techniques – Shimadzu UV-PC 2401 spectrophotometer, Mass spectrophotometer: GC-MS finnigan mat SSQ 7000 Mass spectroscopy 70 ev., Agilent GC -system.6890N. ^{1}H-NMR spectra were recorded in (DMSO-d_6) on Jeol-Ex-270 MHZ spectrophotometer.

Isolation of lipids – About 1.5 kg of air dried powdered aerial parts of plant material *M. peregrina* was extracted in a Soxhlet apparatus with petroleum ether (40-60°C). The combined extracts were filtered through Fuller's earth, and the filtrate was evaporated under vacuum at

40°C. The residue (8.5 g) was dissolved in boiling acetone (350 ml), cooled and left over night. An amorphous white precipitate was formed which was separated by filtration (2.1 g) representing acetone insoluble fraction. The acetone soluble fraction was saponified (N/2 methanolic KOH), and the unsaponifiable matter (1.9 g) was first separated by extraction with ether. The liberated fatty acids mixture, after acidification of the saponifiable matter was extracted with peroxide- free ether, and then methylated (methanol, 5% H_2SO_4). Aliquots of the isolated fatty alcohols, unsaponifiable fraction, and methyl esters of fatty acids were subjected to GLC analysis.

Isolation of Flavonoids – The defatted powdered plant material was extracted with ethanol (70%). The combined alcoholic extract was evaporated *in vacuo*, dissolved in hot distilled water and left overnight in a refrigerator then filtered. The aqueous filtrate was extracted with successive portions of chloroform followed by ethyl acetate. Each of combined solvent extracts was separately evaporated *in vacuo*.

The ethyl acetate fraction (2.8 g) was subjected to column chromatographic fractionation using silica gel (100 g, silica gel, 230-270 mesh, Merck) packed in a glass column (90 cm×4 cm i.d.) eluted with $CHCl_3$ then a gradient of $CHCl_3$/MeOH with an increasing amount of MeOH. The chromatic fractionation was monitored by (TLC GF_{254}, EtOAc:formic acid:acetic acid : water, 30 : 1.5 : 1.5 : 8 v/v). The fraction eluted with $CHCl_3$/MeOH (80 : 20 v/v) was found by TLC (silica gel GF_{254} with the same eluting solvent) to contain two main flavonoidal compounds. It was further purified by preparative paper chromatography using Whatmann 3 MM and *n-butanol*: acetic acid: water (4 : 1 : 5). The upper layer was found to afford compounds 1 and 2 which were eluted with methanol. The fraction eluted with $CHCl_3$: MeOH (70 : 30) was found by TLC, using the same solvent system mentioned before, to contain three compounds. It was further purified using repeated column chromatography on silica gel followed by Sephadex LH-20 column eluted with methanol (70%) to yield compounds 3 and 4.

Animals – Rats of both sexes (150-200 g), and mice (18-20 g), were used in the experiments. Animals were housed under standardized conditions of light and temperature and received standard rat chow and tap water *ad libitum*. Animals were randomly assigned to three different experimental groups. Each group was kept in a separate cage. All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (Canadian Council on Animal Care Guidelines, 1984).

Toxicological study – LD_{50} was determined according to the methods described by Karber (1931) using groups of 10 mice. The symptoms of toxicity and post mortem findings were recorded within the first 24 hrs post-injection.

Induction of Diabetes Mellitus – Diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg b.wt), freshly dissolved in citrate buffer (0.05 M, pH 4.5) (Korthuis *et al.*, 1987). Four days later the blood glucose was assayed and diabetes was verified. Rats were divided into three equal groups (6 rats each). The first group was used as control and injected intraperitoneally (i.p.) by sterile saline (1 ml/100 g b.wt.). Animals of the 2nd and 3rd groups were i.p. injected with daily doses of 1/20 and 1/10 LD_{50} of *M. peregrina* (defatted ethanolic extract) which were equivalent to 56.7 mg and 113.4 mg/kg/d, respectively, for 30 successive days.

Blood samples were collected from retro-orbital venous plexus of each rat in plain test tubes. Serum was prepared for biochemical assay of glucose according to Trinder (1969); transaminases (AST and ALT) according to Reitman and Frankel (1957); alkaline phosphatase according to Kind and King (1954); serum triglycerids and total cholesterol according to Fossatip (1982) and Richmond (1973) respectively. High density lipoprotein (HDL-cholesterol) and low density lipoprotein (LDL-cholesterol) were measured by a kit from Quimica Clinica Aplicade S. A. 43870 (Amposta, Spain, P. O. Box 20).

Tests on inflammation : Carrageenan-induced paw oedema – Paw oedema was induced by sub-plantar injection of 100 ml of 1% sterile carrageenan lambda (in saline) into the right hind paw (Winter *et al.*, 1962). The contralateral paw received an equal volume of saline. The paw volume was determined immediately before carrageenan injection and at selected times thereafter using a plethysmometer (Ugo Basile, Milan, Italy). Oedema was expressed as a percentage of change from the control (pre-drug) value. The effect of *M. peregrina* (56.7 mg and 113.4 mg/kg, 0.2 ml, i.p.) or indomethacin (18 mg/kg, s.c., 0.2 ml) given 30 min before treatment was studied. The control groups received saline (0.2 ml, i.p.) (n = 6).

Tests on analgesia : Acetic acid-induced writhing – Separate groups of 6 rats each were administered the vehicle and/or *M. peregrina* (56.7 and 113.4 mg/kg , 0.2 ml, i.p.). After 30 min interval, an i.p. injection of 0.6% acetic acid was administered (Koster *et al.*, 1959). Each rat was then placed in an individual clear plastic observational chamber, and the total number of writhes made by each rat was counted for 20 min.

: Hot-plate test – The hot-plate test was performed on rats by using an electronically controlled hot-plate (Ugo Basile, Italy) heated to 52°C (± 0.1°C). The cut-off time was 30s. Groups of 6 rats each were given *M. peregrina* (56.7 and 113.4 mg/kg, 0.2 ml, i.p.), saline (control), or indomethacin (18 mg/kg, 0.2 ml, s.c.), 30 min prior to testing. Latency to lick a hind paw or jump out of the apparatus was recorded sequentially before and at 0.5, 1, and 2 h post-treatment.

Tests on behavior : Rota rod testing – Motor performance of male mice (18-20 g) was measured as the latency to fall from an accelerating rota rod located over plates connected to an automatic counter (Ugo Basile, Varese, Italy). After 2-min training period, mice were administered the vehicle (saline) or *M. peregrina* (56.7 and 113.4 mg/kg , 0.2 ml, i.p.) and 30 min later placed on the rotating rod as it accelerated from 4 to 40 rpm over 5 min (Millan *et al.*, 1994). The test was repeated 2 h after vehicle or drug injection.

: Porsolt's forced – swimming test-Each mouse was placed individually in a glass cylinder (diameter 12 cm, height 24 cm) filled with water at a height of 12 cm. and the duration of immobility was measured. Water temperature was maintained at 22-23°C. The floating time, which was the measure of despair (Persolt *et al.*, 1977), was recorded after treatment with M. peregrina (56.7 and 113.4 mg/kg, 0.2 ml, i.p.), saline or amitriptyline (15 mg kg^{-1}, i.p.).

Gastric ulcerogenic studies – Gastric mucosal damage was evoked in rats by the administration of indomethacin (20 mg/kg, s.c.). The effect of *M. peregrina* (56.7 mg and 113.4 mg/kg 0.2 ml, i.p.) administered at time of indomethacin injection was studied. Food and water were provided *ad libitum*. Rats were killed 24 h after drug administration, stomachs excised and examined for gastric mucosal lesions according to Radwan (1967) and Mózsik *et al.*, (1982).

Statistical analysis – Data are expressed as means ± S.E. One and two-way analysis of variance, followed by a Tukey's multiple range test for *post hoc* comparison of group means, were used for analysis of data When there were only two groups a two-tailed Student's t test was used. For all tests, effects with a probability of $P < 0.05$ were considered significant.

Results and Discussion

GLC analysis of the unsaponifiable fraction proved to be a mixture of hydrocarbons, sterols and triterpenes. Identification of the compounds was carried out by comparison of their retention time with the available reference compounds. The identified compounds listed in Table (1) illustrated that β-sitosterol represents the main steroidal component (28.79%).

GLC analysis of the fatty acid methyl esters resulted in the identification of 13 fatty acids (Table 2) in which $C_{16(0)}$ is the major component (24.86). Moreover, it was shown that the saturated fatty acids represent the major constituents of the total mixture (62.25%) and total unsaturated fatty acids (37.75%).

Table 1. GLC analysis of unsaponifiable matter of *M. peregrina*

Peak No.	RRT	Relative %	Constituents
1	0.098	1.77	Dodecane C_{12}
2	0.135	0.55	Tridecane C_{13}
3	0.21	5.85	Tetradecance C_{14}
4	0.306	3.52	Hexadecane C_{16}
5	0.38	1.37	–
6	0.41	2.844	Heptadecane C_{17}
7	0.556	2.401	Octadecane C_{18}
8	0.57	6.37	Nonadecane C_{19}
9	0.59	2.21	Eicosane C_{20}
10	0.65	2.215	Heneicosane C_{21}
11	0.81	5.25	Tricosane C_{23}
12	0.86	2.193	Tetracosane C_{24}
13	0.92	8.99	Hexacosane C_{26}
14	0.95	4.07	Cholesterol
15	1	28.79	β-sitosterol
16	1.05	5.65	Stigmasterol
17	1.08	8.46	Campsterol
18	1.12	6.5	β-amyrine

RRT = Relative to retention time of β-sitosterol (36.51 min).

Table 2. GLC analysis of fatty acids methyl esters of *M. peregrina*

Peak No.	RRT	Relative %	Constituents
1	0.73	3.74	$C_{12(0)}$(lauric)
2	0.86	5.34	$C_{14(1)}$tetradecaenoic
3	0.87	16.43	$C_{14(0)}$Myristic
4	0.91	4.7	$C_{15(0)}$Pentadecanoic
5	1	24.68	$C_{16(0)}$ Palmitic
6	1.05	1.38	$C_{17(0)}$Heptadecanoic
7	1.09	6.20	$C_{18(3)}$Linolenic
8	1.10	13.60	$C_{18(2)}$ Linoliec
9	1.13	2.71	$C_{18(1)}$Oliec
10	1.22	3.57	$C_{19(0)}$Nonadecanoic
11	1.37	6.38	$C_{22(0)}$Docosanoic
12	1.40	7.45	-------
13	1.66	2.45	$C_{24(1)}$Nervonic

RRT = Relative to retention time of palmitic acid $C_{16(0)}$ (20.71 min).

Flavonoidal Components – Compounds 1 & 4 From the uv,spectrum with shift reagents,^{1}H-NMR,and MS they were found to be, : "*Quercetin*" *and* "*Quercetin-3-O-rhamnoglucoside*" (*Rutin*) (Mabry *et al.*, 1970, Markham, 1982)

Compound 2 – The compound showed a brown spot under UV light and changed into greenish yellow on spraying with $AlCl_3$. The chromatographic data in different solvents (0.12 in 15% acetic acid 0.74 in BAW 3 : 1 : 1) proved that it is an aglycone in nature, λ max (MeOH) for band-1 is 331 nm which indicates that it is a flavone type (Mabry *et al.*,1970). A bathochromic shift (66 nm) in band-1 indicates the presence of a free OH group at C-4′. The presence of free OH group at C-7 was confirmed through the bathochromic shift in band-II (8 nm) upon addition of NaOAC. A bathochromic shift of band-I (39 nm) upon addition of AL Cl_3 indicates the presence of C-5 OH group.

^{1}H-nmr (DMSO-d_6) showed signals at δ 8.2, (2 H, s, H-2', 6'), and 7.9, (1H, s, H-3) the protons of four methoxy groups appears as sharp singlets in region between 3.75-3.99 ppm (Benard *et al.*, 2000). The FAB-Mass spectrum (+ve mode) showed a molecular ion peak at *m/z* = 391 which corresponds to the molecular formula $C_{19}H_{18}O_9$ And the fragment ions appeared at *m/z* 153 (ring B) and *m/z* 237(ring A) (Mossa *et al.*,1992). Also Fragment ion peaks at *m/z* 389 (10%, M-1), 361 (5%, M-OCH_3), 277 (90%, M-{2OCH_3 + 2H_2O + CH_3}), 259 (15% {M-2OCH_3 + 3H_2O + CH_3}) from the previous chromatographic and spectroscopic data compound2 could be identified as 6, 8, 3', 5' tetramethoxy apigenin.

Compound 3 – The compound appears as a deep purple spot at R_f 0.28 in 15% acetic acid and R_f 0.35 in BAW 3 : 1 : 1 which indicates its glycosidic nature (as a monoglucoside) (Mabry *et al.*, 1970). The UV spectrum in methanol showed λ max at 275 nm, and 342 nm which is indicative to be a flavone type. The presence of free OH group at C-4′ was confirmed through the bathochromic shift (54 nm with high intensity on addition of NaOMe). The absence of an ortho-dihydroxy system was proved

Table 3. Effect of defatted ethanolic extract of *M. Peregrina* on serum glucose, AST and ALT in diabetic rats

Groups	Time of sampling "days" after beginning of i.p. administration of the extract.											
	Zero time				15 days				30 days			
	Glucose	AST	ALT	AP	Glucose	AST	ALT	AP	Glucose	AST	ALT	AP
Control without treatment	235.0 ± 16.48	62.50 ± 1.88	50.33 ± 2.53	16.0 ± 1.06	213 ± 13.45	58.50 ± 1.93	48.33 ± 2.17	16.83 ± 1.35	206.5 ± 13.55	56.67 ± 2.39	46.83 ± 1.99	14.67 ± 1.02
Extract 5.67 mg/100 g	275.0 ± 23.63	67.50 ± 3.06	49.33 ± 3.09	15.08 ± 1.07	201.67* ± 13.52	54.0** ± 2.82	45.33 ± 2.29	12.33* ± 0.61	68.5** ± 4.35	49.33*** ± 1.93	41.00* ± 1.81	10.33* ± 0.56
Extract 11.34 mg/100 g	277.8 ± 28.96	63.83 ± 1.85	51.33 ± 2.59	14.83 ± 0.95	168.33** ± 10.14	49.33** ± 2.92	36.67** ± 2.47	11.83* ± 0.70	38.17*** ± 5.31	36.5*** ± 1.95	30.17*** ± 1.35	10.83* ± 0.95

* Indicates a statistically significant difference of the value when compared with zero time in the same group (*p < 0.05, **p < 0.01, ***p < 0.001).

Table 4. Effect of defatted ethanolic extract of *M. Peregrina* on serum triglyceride, cholesterol, HDL and LDL in diabetic rats

Groups	Time of sampling "days" after beginning of i.p. administration of the extract											
	Zero time				15 days				30 days			
	TG	Cholesterol	HDL	LDL	TG	Cholesterol	HDL	LDL	TG	Cholesterol	HDL	LDL
Control without treatment	156.67 ± 8.13	159.17 ± 8.98	31.67 ± 1.41	36 ± 1.21	144.17 ± 4.17	150.83 ± 4.90	32.50 ± 1.34	34.50 ± 1.18	136.67 ± 4.77	141.67 ± 5.58	33.00 ± 1.41	32.83 ± 1.45
5.67 mg/100g extract	162.5 ± 8.54	151.17 ± 8.75	32.17 ± 1.30	38 ± 1.15	146 ± 3	135.83 ± 6.64	35.33 ± 1.15	33.83 ± 1.49	135.83* ± 4.55	123.67 ± 9.61	38.83** ± 1.22	30.5** ± 1.82
11.34 mg/100 g extract	169.17 ± 5.83	154.83 ± 6.64	31.67 ± 0.99	38.17 ± 1.08	134.17*** ± 4.90	134.0* ± 6.30	33.17 ± 0.83	32.17** ± 0.83	119.17*** ± 5.23	124.17** ± 4.17	36.83* ± 1.35	27.33*** ± 0.71

* Indicates a statistically significant difference of the value when compared with zero time in the same group (*p < 0.05, **p < 0.01, ***p < 0.001).

where there is no hypsochromic shift in band-1 in $AlCl_3$/HCl spectrum. No bathochromic shift occur in band-II after the addition of NaOAC indicating the absence of free OH group at C-7. The FAB-Mass (+ve mode) spectrum showed a molecular ion peak at *m/z* = 447 and another peak at m/z = 300 indicating the presence of sugar moiety (146 which may be a deoxyhexose). Acid hydrolysis (Mabry *et al.*, 1970). revealed rhamnose as a sugar moiety (by PC and authentics) and chrysoeriol as an aglycone.

The ^{1}H nmr spectrum (DMSO-d_6) displayed signals at δ 7.7 (1H, d, *J* = 2Hz, H.2'), 7.45 (1H, d, *J* = 2Hz, H-6'), 6.9 (1H, d, *J* = 8.5, H-5'), 6.65 (1H, s, H-3), 6.25 (1H, s, H-8), 6. (1H, s, H-6), 3.85 (3H, s, OCH_3) and 4.9 (1H, d, *J*, = 1.5 Hz, H anomeric for rhamnose, 1.1, d. for CH_3 protons of rhamnose (Markham, 1982).

The previous chromatographic and spectroscopic data substantiated that the compound is chrysoeriol-7-0-rhamnoside.

Toxicological study – Symptoms of acute toxicity of defatted alcoholic extract of *M. peregrina* exhibited increased of respiration rate, cyanoses of mucous membranes, general depression characterized by apathy, loss of righting reflex, convulsion and death. The LD_{50} of the defatted alcoholic extract of *M. peregrina* was found to be 113.4 mg/100 g b.wt., LD_{10} and LD_{100} 60 and 192 mg/100 g b.wt., respectively, when intraperitonealy injected in mice.

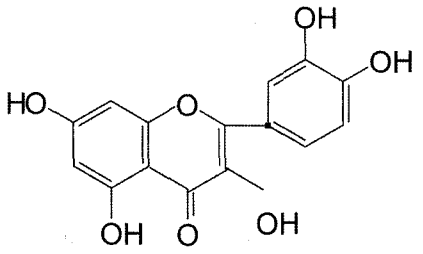
Effect of *M. peregrina* on diabetic rats : Effect on serum glucose levels – *M. peregrina* defatted alcoholic extract caused a significant decrease in serum glucose for two dose levels after 30 days (Table 3). Low doses did not show any effect on serum glucose levels for up to two weeks.

: Effect on AST, ALT, and alkaline phosphatase – *M. peregrina* ethanol extract caused a significant decrease in serum AST and ALT for two dose levels after two and four weeks. Also defatted alcoholic extract of *M. peregrina* induced a significant decrease in alkaline phosphatase for high dose levels after two and four weeks as shown in Table 3.

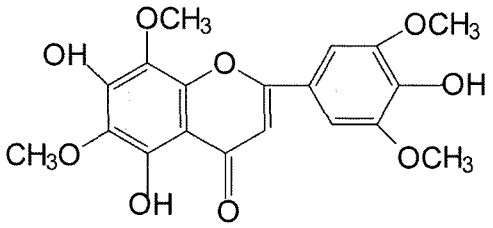
: Effect on lipid components – In diabetic rats the administration of *M. peregrina* ethanolic extract exhibited a significant decrease in serum triglycerides, cholesterol, and LDL levels. On the other hand, *M. peregrina* caused a significant increase in HDL levels as shown in Table 4.

Effect of M. peregrina on carrageenan-induced paw oedema – *M. peregrina* administered i.p., 30 min prior to carrageenan at doses of 56.7 and 11.34 mg/kg significantly inhibited the paw oedema response (Fig. 1). The percentages of inhibition by *M. peregrine* (56.7 & 113.4 mg/kg) were 44.4, 41.2, 40.3, 41.2% and 55.8, 57.3, 55.3, 51.9% at 1, 2, 3 and 4 h post-carrageenan, respectively. In comparison, the positive control, indomethacin markedly and significantly inhibited the paw oedema response by 56.5, 60, 62.9, and 60.8% at 1, 2, 3 and 4 h after carrageenan, respectively.

Chemical Structures of Isolated Flavonoids:

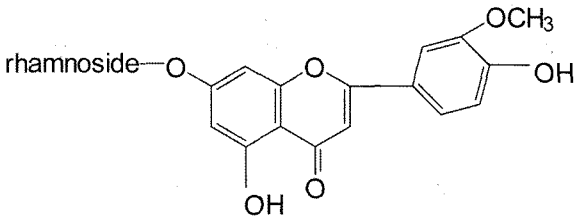


Compound 1 (Quercetin)



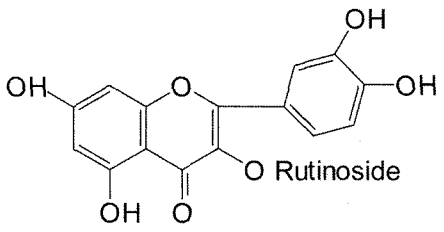
Compound 2

6,8,3',5',tetramethoxy Apigenin



Compound 3

Chrysoeriol-7-O-rhamnoside



Compound 4

Quercetin-3-O-rutinoside (rutin)

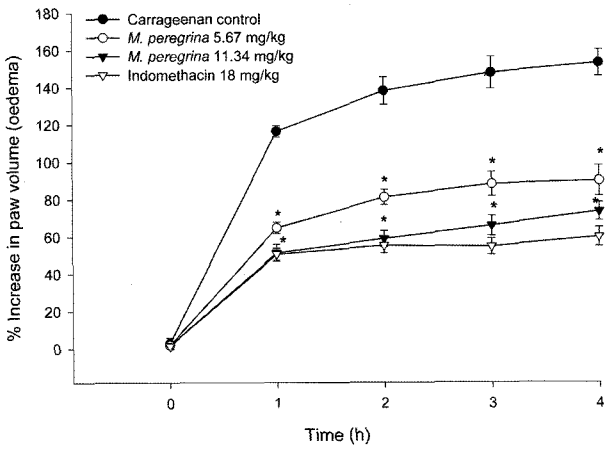


Fig. 1. Effect of *M. peregrina* extract (in two dose levels) on rat paw oedema-induced by carrageenan and compared to the effect of indomethacin. Six rats were used per each group *P < 0.05 compared with control at corresponding time point.

Effect of *M. peregrina* on analgesia : Hot-plate test – The mean reaction time on the hot plate was significantly delayed after the administration of *M. peregrina* (39.8 and 55.4% reduction by 56.7 and 113.4 mg/kg) or indomethacin 18 mg/kg (61.3% reduction), compared with basal values, denoting decreased pain perception ($P < 0.05$, one way ANOVA). (Fig. 2).

: Acetic acid-induced writhing – Acetic acid-induced writhing was significantly reduced in mice receiving *M. peregrina*. The antinociceptive activity of the drug was dose-related with a maximal reduction of the writhing score of 45.6 and 70.7% by 56.7 mg and 113.4 mg/kg of *M. peregrina*, respectively (Fig. 3). The analgesic effect of 113.4 mg/kg of *M. peregrina* was significantly higher than that of the positive control indomethacin which inhibited the writhing response by 56% (P < 0.05).

Effect of *M. peregrina* in behavioral tests : Rotarod testing – *M. peregrina* (56.7 and 113.4 mg/kg) did not produce any significant change on the rotarod performance of mice. Both controls and trimetazidine-treated mice remained on accelerating rotarod during the acceleration period (5 min) and for 5 min thereafter (data not shown).

: Porsolt's forced-swimming test – *M. peregrina* 56.7 and 113.4 mg/kg did not reduce immobility time in contrast to the ricyclic antidepressant drug ,imipramine, which significantly reduced immobility time (Fig. 4).

Effect of *M. peregrina* on gastric mucosal lesions caused by indomethacin – *M. peregrina* (11.34 mg/kg) administered at time of indomethacin injection inhibited the development of gastric lesions (Fig. 5).

In the present study, the toxic effects of the defatted alcoholic extracts of *M. peregrina* was studied. Post

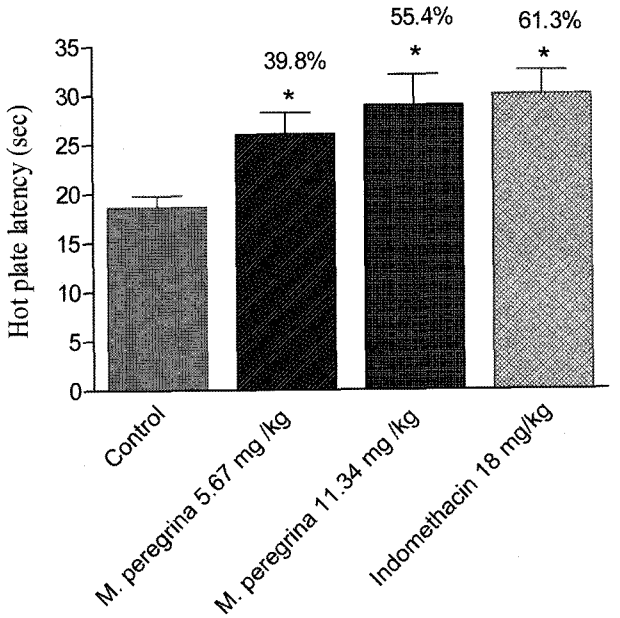


Fig. 2. Hot plate latency (seconds) of control and *M. peregrina*-treated rats. *P < 0.05 compared to control. Six rats were used per each group.

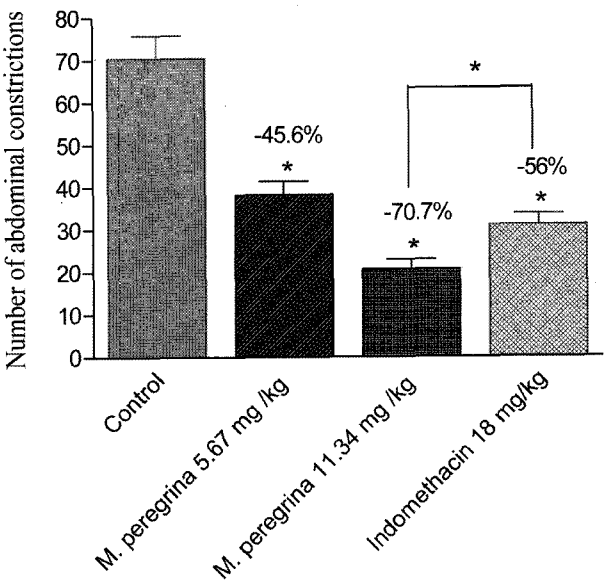


Fig. 3. Number of writhings (abdominal constrictions) of control and *M. peregrina*-treated mice. Six mice were used per each group. *P < 0.05 compared to control and between indomethacin and the large dose of the extract.

mortem examination revealed congestion of internal organs and the change of blood color to dark brown that may suggest the oxidation of oxyhaemoglobin to methea-moglobin by the toxic constituents of the extract (Murray *et al.*, 1993). On the other hand, the present results revealed that the administration of defatted alcoholic extract of *M. peregrina* caused a significant decrease in

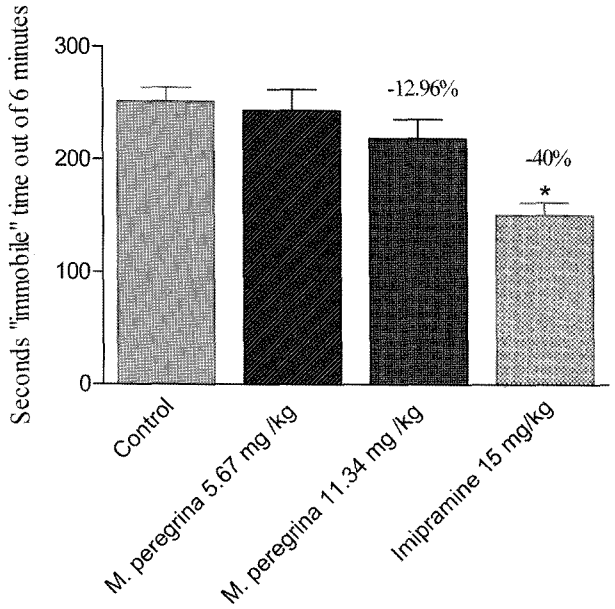


Fig. 4. Immobility time (seconds) of control and *M. peregrina*-treated mice subjected to Porsolt's forced swimming test. Six rats were used per each group. *P < 0.05 from control.

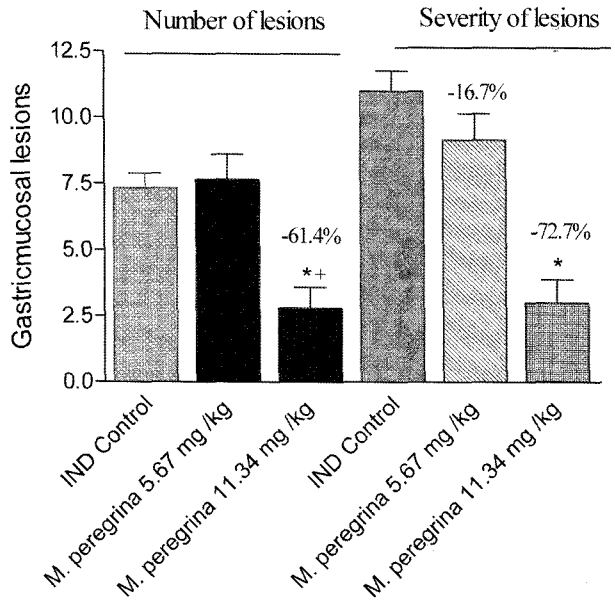


Fig. 5. Effect of two doses of *M. peregrina* on the number and severity of gastric lesions induced by indomethacin (IND) in rats. Results are expressed as means of 6 observations ± SE. *P < 0.05 from IND control.

serum glucose, liver enzymes and lipid profile in diabetic rats which are in agreement with many published results (Ghasi *et al.*, 2000; Ashok-Kumar & Pari 2003 ; Kar *et al.*, 2003 ;Mehta *et al.*, 2003).

Antioxidants have been used with success in the management of several hepatic disorders. *M. peregrina*, a medicinal plant used in folk medicine for the treatment of a variety of medical conditions possesses hypoglycemic, antioxidant and hypolipidemic properties. The major bioactive compounds of defatted alcoholic extract of *M. peregrina* were found to be flavonoid compounds such as quercetin and rutin. Other antioxidant flavonoids suppress oxidation and cytotoxicity of LDL *in vitro* (Furst 1995).

The main function of these compounds are antioxidant activity (Siddhuraju & Becker, 2003). On the basis of the results obtained, *M. peregrina* was found to be a potential source of natural antioxidants. Supplementation of antioxidants may be a protective factor against free radical-induced beta cell damage (El-Wakkad *et al.*, 2000), thus preventing or ameliorating diabetes mellitus. Glombitza *et al.* (1993) and Ferrel *et al.* (1979) also suggested that flavonoids inhibit cAMP phosphodiesterase. cAMP is a modulator of insulin secretion. Furthermore, *M. peregrina* can improve glucose metabolism (Mehta *et al.*,2003) as well as the overall condition of the diabetic patients not only by its direct hypoglycemic effect but also by improving lipid metabolism and liver function (Furst, 1995; Siddheraju and Becker, 2003).

The results obtained in the present study also provide evidence that defatted alcoholic extract of *M. peregrine* possesses anti-inflammatory and analgesic activities. These activities were similar to those of indomethacin. Moreover, the extract reduced the intensity of the peritoneal inflammation produced by acetic acid in mice, indicating its ability to inhibit the permeability of the small blood vessels. These results suggest that the anti-inflammatory activity of *M. peregrina* may involve an inhibitory effect on autacoids (histamine, serotonin, kinins or prostaglandins) or a stabilizating effect on lysosomal membranes. It has been suggested that bioactive compounds such as flavonoids act as anti-inflammatory agents (Alcaraz and Jimenez, 1988) and that the anti-inflammatory properties are a consequence of their inhibitory action on arachidonic acid metabolism, as demonstrated *in vitro* and *in vivo* (Alcaraz and Jimenez, 1988). Bioactive flavonoids that have been found in *M. peregrina* may be responsible for its anti-inflammatory activity. The main side effect of non-steroidal anti-inflammatory drugs is their ability to produce gastric lesions (Shah *et al.*, 1999). *M. peregrina* administered at time of indomethacin injection inhibited the development of gasric lesions. Therefore, the potential medicinal value of *M. peregrina* as hypoglycemic agent, hypolipidemic, antioxidant, anti-inflammatory, and analgesic effects without side effects on gastric mucosa and motor activity. More studies are needed to throw light on *M. peregrina* extract for economic clinical utilization of this medicinal plant.

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