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Anti-inflammatory and Antihepatotoxic activities of the Roots of *Moringa pterygosperma* Gaertn

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The roots of *Moringa pterygosperma* Gaertn. *(Moringaceae)* in powder and extract form were studied for their anti-inflammatory and antihepatotoxic activities. Among these, the methanolic extract showed significant (P<0.01) oedema suppressant activity against carrageenan-induced paw oedema similar to that of indomethacin. The aqueous extract against carbon tetrachloride, total aqueous extract against paracetamol and methanolic extract against rifampicin treatments showed significant (P<0.01) antihepatotoxic activity.

THE roots of *Moringa pterygosperma* Gaertn. (*Moringaceae*) are carminative, stomachic, abortifacient, cardiac tonic and used in paralytic conditions, rheumatism, spasm of the bowel, hysteria, ascites and enlargement due to diseases of the liver and spleen[1,2], dropsy, gout, asthma and epilepsy. The root bark contains alkaloids moringine and moringinine[3], essential oil, phytosterols, waxes, resins and an antibiotic principle, pterygospermin[4] possessing antifertility activity. In the present investigation, the powder and different extracts of the roots of *Moringa pterygosperma* were studied for antiinflammatory and antihepatotoxic activities in albino rats.

EXPERIMENTAL

Plant Material:

The roots of *Moringa pterygosperma* were purchased from the local market and their identity was confirmed by comparing with herbarium specimen preserved in the museum of Botany Departments of M.S. University of Baroda, Vadodara, Gujarat and Central Drug Research Institute, Lucknow, India. The roots, dried and reduced to 60 mesh powder, were extracted with methanol (5.24%) using a soxhlet extractor. A successive aqueous (9.31%) and total aqueous (13.43%) extracts were prepared by decoction method. All these extracts were dried using a rotary flash evaporator at 50°. The powder (120 mesh) and these extracts were screened for biological activities.

Pharmacological screening:

5% w/v acacia mucilage (1 ml/kg, p.o.) was used as a vehicle. Indomethacin (20 mg/kg, p.o.), ibuprofen (100 mg/kg, p.o.), ketoprofen (20 mg/kg, p.o.), paracetamol (3 g/kg, p.o.), rifampicin (1 g/kg p.o.) were used as standard drugs. CCl_4 was obtained from BDH and E.Merck (I) Ltd. Bombay and was employed as a 1:1 solution in olive oil at a dose of 2.5 ml/kg, p.o.. Carrageenan (Sigma. Co., U.S.A.) 1% w/v suspension in saline was prepared and 0.1 ml per rat was injected into the right hind paw, to induce paw oedema. All the extracts and the powdered drug were administered at a dose of 100 and 500 mg/kg, p.o., respectively.

Wistar strain albino rats (150-200 g) of either sex maintained under standard laboratory conditions (temperature:23±2°, relative humidity: 55±10% and 12 h

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light and dark cycle) were used throughout the study. The rats were allowed standard laboratory feed and water *ad libitum*. They were devided randomly into groups of six rats each before initiating various pharmacological studies.

Toxicity studies:

The rats were divided into control and test groups[5]. The control group received the vehicle while the test groups received different test substances orally.

Acute Toxicity

The test groups received different test suspensions in 0.1, 0.5, 1.0, 2.0, 5.0, 7.0, and 10.0 g/kg, p.o. and were observed for any mortality till 48 h.

Hepatotoxicity

The test groups received various test suspensions in 100 mg/kg, p.o. in three divided doses at 12 h interval. After 12 h of last dose administration, blood, collected by puncturing the retroorbital plexus, was allowed to coagulate at 37° for 30 min. The serum, separated by centrifugation at 2500 rpm, was analysed for various biochemical parameters.

Anti-inflammatory activity:

These studies were carried out in male albino rats against carrageenan - induced hind paw oedema[5]. All the suspensions were administered orally 30 min before the injection of carrageenan and percent inhibition of oedema at different time intervals was calculated.

Antihepatotoxic activity:

The rats were divided into control, hepatotoxin treated and test (test sample + hepatotoxin) groups. The control and hepatotoxin groups received the vehicle whereas the test groups received test samples orally. The test groups received the first dose of respective test suspension 30 min before the administration of a single oral dose of the hepatotoxin.

CCl_4 - induced hepatotoxicity[5]

The plant extract suspensions were administered orally three times at 12 h interval. A single dose of CCl_4 (2.5 ml/kg, p.o.) was administered 30 min after the first dose of plant extract suspensions. After 36h of CCl_4 administration, 2 to 3 ml of blood was collected by puncturing the retroorbital plexus and was allowed to clot for 45 min at room temperature. Serum, separated by centrifugation at 2500 rpm 15 min, was used for the estimation of various biochemical parameters.

Paracetamol - induced hepatotoxicity[6]

A single oral dose of plant extract suspensions was administered daily for 3 days to the test group animals. On the 3rd day of the treatment, a single oral dose of paracetamol (3 g/kg) was administered to paracetamol treated and test groups, 30 min after the third dose of plant extracts. After 48 h of paracetamol administration, blood was collected and serum was analysed for various biochemical parameters.

Rifampicin - induced hepatotoxicity[6]

Four doses of the plant extract suspensions were administered orally four times at 12 h interval. A single oral dose of rifampicin (1 g/kg) was administered 30 min after the first dose of plant extracts. After 48 h of rifampicin administration, blood was collected and serum was analysed for various biochemical parameters.

Assessment of liver function:

Biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) were determined according to the method described by Reitman and Frankel[7]. Alkaline phosphatase (ALKP) was determined according to Szasz method[8]. Total bilirubin (TBil), and direct bilirubin (DBil) were measured according to the method of Jendrassik and Grof[9].

Statistical analysis:

The mean value ± SEM was calculated for each parameter[10]. Percent reduction in biochemical parameters by the test samples against the hepatotoxin was calculated by considering the differences in biochemical parameters between the hepatotoxin-treated and the control group as 100 % level of reduction. For determination of significant intergroup differences each parameter was analysed sepafately and one way analysis of variance was carried out. Individual comparison of group mean values were done using Dunnett's test[11].

Table.1 Effect of the roots of *Moringa pterygosperma* on Carrageenan-induced paw oedema

Group	Mean differences in Paw Volumes (ml) ± SEM				
	1 h	2 h	3 h	4 h	5 h
Control	0.43±0.01	0.68±0.04	0.73±0.02	0.75±0.03	0.77±0.02
Indo-methacin	0.32±0.02* (25.6)	0.34±0.02* (50.0)	0.42±0.03* (42.5)	0.50±0.04 (33.3)	0.55±0.03* (28.6)
Ibuprofen	0.20±0.00** (53.5)	0.32±0.01* (52.9)	0.44±0.01* (39.7)	0.53±0.03 (29.3)	0.58±0.02* (24.7)
Ketoprofen	0.21±0.02** (51.2)	0.22±0.02** (67.7)	0.24±0.02*** (67.1)	0.27±0.03 (64.0)	0.39±0.03** (49.4)
Powder	0.49±0.02 (-)	0.79±0.02 (-)	0.90±0.02 (-)	1.09±0.04 (-)	1.12±0.04 (-)
Methanolic extract	0.16±0.02** (62.8)	0.22±0.02** (67.7)	0.23±0.05* (68.5)	0.38±0.03* (49.3)	0.48±0.03* (37.7)
Aqueous extract	0.45±0.01 (-)	0.67±0.01 (1.5)	0.75±0.02 (-)	0.78±0.03 (-)	0.81±0.02 (-)
Total aq. extract	0.43±0.01 (-)	0.78±0.01 (-)	0.91±0.02 (-)	0.96±0.02 (-)	0.98±0.03 (-)

Significant reduction compared to: Control=, Indomethacin=**, Ibuprofen=***, ketoprofen=**** (P<0.01) Numbers in Parentheses indicates % reduction in paw oedema (-) = No reduction*

Table.2 Effect of the roots of *Moringa pterygosperma* on CCl_4 - induced hepatotoxicity

Group	Biochemical Parameters Mean ± SEM				
	SGPT (U/ml)	SGOT (U/ml)	ALKP (U/l)	T Bil (mg/dl)	D Bil (mg/dl)
Control	56.96±3.24	137.53±12.28	169.06±4.66	1.23±0.03	0.20±0.01
CCl_4	725.51±38.03	1160.77±52.08	456.70±13.15	3.55±0.11	1.50±0.03
Powder	138.12±2.04* (87.86)	247.85±3.55* (89.22)	168.55±5.84* (100.17)	1.06±0.02* (107.33)	0.21±0.01* (99.23)
Methanolic extract	49.67±1.70* (101.09)	37.22±0.99* (109.80)	146.73±2.08* (107.76)	1.23±0.02* (100.00)	0.53±0.01* (74.62)
Aqueous extract	67.26±1.71* (98.46)	27.78±1.21* (110.73)	135.97±2.14* (111.50)	1.27±0.01* (98.28)	0.31±0.01* (91.54)
Total aq. extract	195.82±2.00* (79.23)	748.54±5.24* (40.29)	145.08±1.81* (108.33)	1.04±0.03* (108.19)	0.48±0.01* (78.46)

*Significant reduction compared to Carbon Tetrachloride = * (P<0.01)*
Numbers in Parentheses indicates % reduction in biochemical parameters

Table.3 Effect of the roots of *Moringa petrygosperma* on Paracetamol-induced hepatotoxicity

Group	Biochemical Parameters Mean ±SEM				
	SGPT (U/ml)	SGOT (U/ml)	ALKP (U/l)	TBil (mg/dl)	DBil (mg/dl)
Control	58.98±0.63	137.53±1.27	182.67±0.79	0.88±0.02	0.25±0.01
Paracetamol	265.28±3.14	356.00±5.17	313.49±7.40	3.42±0.17	0.57±0.03
Powder	241.64±6.64 (11.46)	291.62±3.62 (29.47)	111.60±3.95* (154.33)	1.26±0.03* (85.04)	0.21±0.01* (112.50)
Methanolic extract	140.35±1.03* (60.56)	327.74±2.85 (12.94)	119.83±1.14* (148.04)	1.23±0.01* (86.22)	0.44±0.01* (40.63)
Aqueous extract	42.31±0.38* (108.08)	157.23±1.92* (90.98)	100.53±1.61* (162.79)	1.43±0.02* (78.35)	0.48±0.01* (28.13)
Total aq. extract	108.00±3.06* (76.24)	129.03±1.86* (103.89)	146.43±3.05* (127.70)	1.02±0.02* (94.49)	0.28±0.01* (90.63)

Significant reduction compared to Paracetamol = *, (P< 0.01)

Numbers in Parentheses indicates % reduction in biochemical parameters

Table.4 Effect of the Roots of *Moringa petrygosperma* on rifampicin-induced hepatotoxicity

Group	Biochemical Parameters Mean ±SEM				
	SGPT (U/ml)	SGOT (U/ml)	ALKP (U/l)	T Bil (mg/dl)	D Bil (mg/dl)
Control	76.24±1.61	85.69±2.16	76.17±1.66	1.01±0.03	0.19±0.01
Rifampicin	195.53±3.50	265.46±2.27	141.05±2.91	2.81±0.05	1.21±0.03
Powder	34.47±1.69* (135.02)	133.90±3.49* (73.18)	63.17±2.05* (120.04)	1.42±0.06* (77.22)	0.84±0.03* (36.27)
Methanolic extract	61.53±0.48* (112.33)	196.62±4.18* (38.29)	63.07±0.81* (120.19)	1.35±0.01* (81.11)	0.64±0.01* (55.88)
Aqueous extract	76.24±1.09* (100.00)	278.00±1.18 (-)	71.67±0.71 (106.94)	1.49±0.01 (73.33)	0.82±0.01 (38.24)
Total aq. extract	49.35±1.69 (122.54)	165.95±1.22 (55.35)	53.93±1.21 (134.28)	2.03±0.03 (43.33)	1.15±0.01 (5.88)

Significant reduction compared to Rifampicin = *, (P<0.01)

Numbers in Parentheses indicated % reduction in biochemical parameters (-) = No reduction

RESULTS AND DISCUSSION

The roots of *Moringa pterygosperma* were selected for further investigations since they have been reported to be of extensive use in Indigenous systems of medicine.

The powder and different extracts were found to be practically non toxic. A dose of 500 mg/kg, p.o. of the powdered drug and 100 mg/kg, p.o. of the extracts were found to produce no significant ($P<0.01$) elevation in the serum biochemical parameters in normal rats indicating their non hepatotoxic nature.

Out of all the samples tested, only the methanolic extract showed significant ($P<0.01$) oedema suppressant activity similar to that of indomethacin (Table.1). The difference in the activity of the methanolic and total aqueous extracts may be due to the degradation of some of the active constituents during the process of extraction.

The aqueous extract followed by methanolic extract, powdered drug and the total aqueous extracts showed significant reduction ($P<0.01$) in all the elevated serum biochemical parameters against carbon tetrachloride treatment (Table.2).

The total aqueous extract showed the maximum significant ($P<0.01$) reduction in all the serum biochemical parameters against paracetamol intoxication. This was followed by aqueous extract, powdered drug and methanolic extract (Table.3).

In case of rifampicin-induced hepatotoxicity, the methanolic extract showed the maximum antihepatotoxic activity followed by the powdered drug, total aqueous and aqueous extracts (Table.4).

Thus the roots of *Moringa pterygosperma* can be used as a potential alternative to various herbal drugs that are utilised in different commercial polyherbal formulations used for antirheumatic and antihepatotoxic activities.

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