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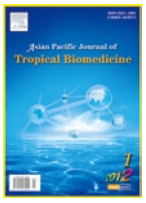

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Antiallergic and analgesic activity of *Momordica dioica* Roxb. Willd fruit seed

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ABSTRACT

Objective: To evaluate the antiallergic and analgesic activities of seed of *Momordica dioica* Roxb. (*M. dioica*) **Methods:** Petroleum ether, ethyl acetate, methanol and aqueous extracts of *M. dioica* seed were screened for anti-allergic activity (200 mg/kg (i.p.) in mice body wt.) and analgesic (dose dependant 50 mg/kg and 100 mg/kg in mice body wt.). **Results:** Methanol extract of *M. dioica* seed shows more significant antiallergic activity ($P<0.01$) as compare to other extracts in all three models of milk induced leukocytosis, milk induced eosinophilia and differentia leukocytes count in mice. In dose dependant analgesic activity petroleum ether and methanol extracts gives significant hot plate activity ($P<0.05$, $P<0.01$) and in acetic acid induced writhing test significantly ($P<0.01$) reduced the number of writhing. **Conclusions:** The antiallergic activity may be due to phytochemical constituents such as polar constituents and flavonoids. The extract possesses analgesic activity which may be due to the phytochemical constituents of the plant.

1. Introduction

Use of plant products is increasing in many segment of the population [1]. At present, thousands of plant metabolites are being successfully used for the treatment of variety of diseases. According to an estimate, 80% of the world's population relied upon plants for their medication [2]. The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products [3]. *Momordica dioica* (*M. dioica*) is a pereannial dioiceous climber found throughout India [4]. *M. dioica* belongs to the family Cucurbitaceae [5]. Folk medicinally *M. dioica* climbing creeper plant is used both in the prevention and cure of various diseases and in the food of humans like Cure vata, biliousness, asthma, leprosy, bronchitis, fever, tumors, tridosha, urinary discharges, excessive salivation, troubles of the heart, inflammation, errhine effect [6]. Anti-allergic, bronchial asthma, antimalarial [7] and antiallergic activity of methanol extract of *M. dioica* fruit pulp [8]. The fruits pulp [9, 10] and root [11] used in the treatment of analgesic.

2. Materials and methods

2.1. Plant material

Fruits of *M. dioica* were collected from Therla, Ta. Patoda, Beed district of Maharashtra, India in September 2009 and authenticated by P.G. Diwakar, Botanical Survey of India, Pune, where a sample specimen (voucher number: RAMAM1) No. BSI/WRC/Tech/2009/593 has been deposited.

2.2. Preparation of extracts

Shade dried, cleaned from extraneous materials, mechanically grinded and coarsely powdered fruit seed of *M. dioica* was subjected to successive solvent extraction in Soxhlet extractor using petroleum ether, ethyl acetate, and methanol as solvent and the marc left was refluxed with water. All the extracts were vacuum dried to produce petroleum ether (1.5%), Ethyl acetate (2.6%), Methanol (18%)

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and Aqueous (4.67%) extracts respectively.

2.3. Animals

Albino mice (Swiss strain) weighing 25-30g either sex were obtained from the Serum lab, Pune. The animals were maintained at room temperature of (25±2) ℃ with relative humidity of (75±5)% under 12 hours dark and light cycle. The animals maintained under standard husbandry conditions and had free access to diet and water. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups each consist of six animals and were fasted overnight prior to the experiments. The Animal Ethical Committee of the institute approved the protocol for the experimental study (AVCOP/32/2009-10).

2.4. Drugs and chemicals

The following drugs and chemicals were used. Chemicals: petroleum ether (60-80 ℃) (RFCL Ltd, India), ethyl acetate (RFCL Ltd, India), methanol (MERCK Ltd, India) and DMSO (Research Lab Industries, India). Drugs: Acetic acid (SD fine chemicals Limited, Mumbai), Paracetamol (Glaxo smith kine Pharma Ltd., Mumbai), Pentazocine (Pure Pharma Ltd., Mumbai) purchased from commercial source.

2.5. Acute toxicity study

Acute toxicity study was carried out as per the guidelines set by the Organization for Economic Co-operation Development (OECD guideline 425) received from the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA). Animals were divided into six groups (n=6). The animals were fasted for overnight with free access to food and water. The petroleum ether, ethyl acetate, methanolic and aqueous extracts was administered orally in doses of 500 mg/kg and 1 000mg/kg of body weight to different groups of mice and observed over 14 days for mortality and physical of behavioral changes [12].

2.6. Antiallergic activity

2.6.1. Milk induced leucocytosis

Mice were divided into five groups, six animals in each group. Animals belonging to group-I received distilled water (DW) 10 mL/kg, (*p.o.*). Animals belonging to group I, II, III, IV and V received boiled and cooled milk injection in dose of 4 mL/kg (*s.c.*). Animals belonging to groups II to V received petroleum ether, ethyl acetate, methanol and aqueous extracts of *M. dioica* fruit seed (200 mg/kg, *i.p.*) respectively, 1 hr before milk injection. Blood samples were collected from each mouse from the retro orbital plexus, under light ether anesthesia. Total leukocyte count was done in each group before drug administration and 24 hr after milk injection. Difference in total leukocyte count before and 24 h. after drug administration was calculated [13].

2.6.2. Milk induced eosinophilia

Mice were divided into five groups, six animals in each group. Animals belonging to group-I received distilled water (DW) 10 mL/kg, (*p.o.*). Animals belonging to group I, II, III, IV and V received boiled and cooled milk injection in dose of 4 mL/kg (*s.c.*). Animals belonging to groups II to V received petroleum ether, ethyl acetate, methanol and aqueous extracts of *M. dioica* fruit seed (200 mg/kg, *i.p.*) respectively, 1 hr before milk injection. Blood samples were collected from each mouse from the retro orbital plexus, under light ether anesthesia. Eosinophil count was done in each group before drug administration and 24 hr after milk injection. Difference in eosinophil count before and 24 h. after drug administration was calculated [14].

2.7. Analgesic activity

Two standard methods *viz.* acetic acid induced writhing reflex and hot plate methods were employed to determine the analgesic activity.

2.7.1. Acetic acid induced writhing-reflex method in mice

The analgesic activity was determined by acetic acid induced writhing method using six albino mice (25-30 g) of either sex selected by random sampling technique. Standard drug Paracetamol (50 mg/kg) and the extracts (50 mg/kg and 100 mg/kg)) were given intraperitoneally 30 minutes prior to the administration of the writhing agent (0.6%v/v aqueous acetic acid, 10 mL/kg). The number of writhings produce in the animal was observed for 30 minutes. The number of writhing and stretching was recorded and compared with the control drug. The percent was calculated using the following ratio [15].

% of protection = (Control mean- treated mean) ×100/ Control mean.

2.7.2. Hot plate method in mice

Mice of either sex weighing between 25-30 g were kept on a hot plate (55±0.5 ℃), the time for forepaw licking or jumping was taken as the reaction time. Mice showing reaction time between 3-5 sec. were selected. Animals not responding in this period were discarded. The reaction time was recorded at 0, 30, 60, 90, 120, 180 minutes following administration of the test compounds or the standard drug (Pentazocine 10 mg/kg *s.c.*) to determine the onset and duration of action. One hour after the administration of vehicle, standard drug and extracts treated mice were individually placed on the hot plate of the analgesiometer maintained at 55 ℃. Analgesic activity was determined by comparing with the control group [16].

2.8. Phytochemical screening of active extracts

Various phytochemical studies including test for carbohydrates, proteins, amino acids, steroid, alkaloids, glycosides and flavonoids compounds were carried out [17].

2.9. Statistical analysis

The results are expressed as mean±SEM. The Dunnett's test was used to make a statistical comparison between groups. Result with $P<0.01$ and $P<0.05$ were considered significant.

3. Results

M. dioica climbing creeper plant fruit seed extracts used in the treatment of allergy and analgesic for different types of models in mice and they shows following results.

3.1. Acute toxicity

From the acute toxicity study, the LD cut-off dose for extracts was found to be 1 000 mg/kg body weight. Hence, the therapeutic doses were taken as 50 mg/kg and 100 mg/kg body weight.

3.2. Antiallergic activity

Table 1
Effect of *M. dioica* seed extracts on milk induced leukocytosis in mice (200 mg/kg).

Groups	Treatment	Number of leukocytes (Cu.mm.)		
		Before treatment	After treatment	Difference
I	Control	6485.00±20.27	12325.00±70.05	5840.00±5.07
II	Petroleum ether	6198.00±105.02	3345.00±312.68**	2819.00±227.43**
III	Ethyl acetate	5683.00±237.80	3529.00±151.33**	2147.00±66.221**
IV	Methanol	9371.00±167.52**	2940.00±202.32**	6630.00±46.94**
V	Aqueous	6793.00±153.36	2483.00±105.88**	4300.00±149.62**

All values are expressed as mean±SEM of a sample size of n=6; level of significance chosen was **$P<0.01$. All treated groups were compared with control group. (One way ANOVA followed by Dunnett's test).

Table 2
Effect of *M. dioica* seed extracts on milk induced eosinophilia in mice (200 mg/kg)

Groups	Treatment	Number of eosinophilia (Cu.mm.)		
		Before treatment	After treatment	Difference
I	Control	492.00±4.10	542.00±21.93	49.00±15.45
II	Petroleum ether	83.67±8.96*	95.67±8.96*	11.67±0.21*
III	Ethyl acetate	66.33±5.49*	132.30±15.34*	69.33±8.10
IV	Methanol	193.70±4.20*	63.33±4.20*	130.30±0.21*
V	Aqueous	74.33±7.02*	128.70±13.40*	54.33±6.39

All values are expressed as mean±SEM of a sample size of n=6; level of significance chosen was *$P<0.01$. All treated groups were compared with control group. (One way ANOVA followed by Dunnett's test)

Table 3
Effect of *M. dioica* seed extracts on differential leukocytes count.

Groups	Treatment	Differential leukocyte count (%)		
		Neutrophils	Lymphocytes	Eosinophils
I	Control	7.67±0.56	5.00±0.37	2.00±0.00
II	Petroleum ether	4.00±0.73**	4.00±0.37	2.00±0.00
III	Ethyl acetate	3.67±0.56**	4.30±0.92	2.00±0.00
IV	Methanol	7.00±0.73	13.00±0.73**	1.33±0.21**
V	Aqueous	8.67±0.56	11.00±0.37**	2.00±0.00

Level of significance chosen was *$P<0.01$. All treated groups were compared with control group

Table 4
Analgesic activity (writhing reflex method) of *M. dioica* fruit seed extracts in mice.

Sr.No.	Treatment	Dose (mg/kg)	Writhings±SEM	% Protection
1	Control	--	26.83±0.95	--
2	Standard	50	8.00±0.26	70.18
3	Petroleum ether	50	16.83±0.31*	38.46
		100	12.00±0.26*	53.85
4	Ethyl acetate	50	22.83±0.54*	15.38
		100	18.50±0.34*	30.77
5	Methanol	50	25.00±0.45	03.85
		100	23.00±0.26*	11.54

*$P<0.01$ compared to control group.

Table 5
Analgesic activity (hot plate method) of *M. dioica* fruit seed extracts in mice.

Sr.No.	Treatment	Dose (mg/kg)	Reaction intervals (Seconds) at time (min)					
			00	30	60	90	120	180
1	Control	--	6.75±0.25	5.75±0.25	5.75±0.75	4.50±0.96	3.50±0.66	3.50±0.65
2	Standard	50	7.00±0.41	9.00±0.71*	9.75±0.47**	6.50±0.65	3.00±0.00	3.00±0.000
3	Petroleum ether	50	6.50±0.29	7.00±0.00	7.25±0.25	6.25±0.85	4.00±1.08	3.50±0.86
		100	6.25±0.76	6.75±0.48	11.00±1.47**	3.25±1.25	4.00±0.48	4.00±0.40
4	Ethyl acetate	50	7.50±1.44	7.00±1.35	8.50±1.26	4.00±0.41	3.75±0.75	3.50±0.50
		100	8.25±1.03	10.25±1.11*	7.00±0.00	3.25±0.25	4.00±0.71	3.00±0.70
5	Methanol	50	7.50±0.29	7.75±0.63	7.50±0.87	5.00±1.80	3.00±0.71	2.70±0.47
		100	8.50±1.56	6.50±0.50	5.25±0.48	3.25±0.63	3.00±0.00	2.50±0.28

*P<0.05, ** P<0.01 compared to control group.

3.2.1. Milk induced leukocytosis in mice

Subcutaneous injection of milk in dose of 4 ml/kg, produced a significant (P<0.01) increase in the leukocyte count after 24 hr of its administration. Mice pretreated with petroleum ether, ethyl acetate, methanol and aqueous extract of *M. dioica* fruit seed have exhibited significant difference in total leukocytes before and after drug treatment. Methanol extract of *M. dioica* fruit seed has highly inhibited the milk induced leukocytosis (P<0.01) with dose of 200 mg/kg (i.p.) as compare to other extracts (Table 1).

3.2.2. Milk induced eosinophilia in mice

Subcutaneous injection of milk in dose of 4 mL/kg, produced a significant (P<0.01) increase in the eosinophil count after 24 hr of its administration. Mice pretreated with petroleum ether, ethyl acetate, methanol and aqueous extract of *M. dioica* fruit seed have exhibited significant difference in eosinophil count before and after drug treatment. Methanol extract of *M. dioica* fruit seed has highly inhibited the milk induced eosinophilia (P<0.01) with dose of 200 mg/kg (i.p.) as compare to other extracts (Table 2).

3.2.3. Milk induced differential leukocyte count in mice

Bronchial asthma is characterized pathologically by an infiltration of eosinophils into the airway submucosa [18]. Methanol extract of *M. dioica* fruit seed showed highly significant inhibited the milk induced eosinophilia (P<0.01) with dose of 200 mg/kg (i.p.) as compare to other extracts. The result showed significant (P<0.01) i.e. up to 1.33% decrease in eosinophils count. But there is increased i.e. up to 23.33% of lymphocytes count. It indicates that increased leukocyte count in total leucocytes count model, mainly due to increased lymphocyte (B and T cells) count (Table 3).

3.3. Analgesic activity

3.3.1. Acetic acid induced writhing-reflex method in mice

At doses 50 and 100 mg/kg i.p. of *M. dioica* fruit seed petroleum ether, ethyl acetate and methanol extracts showed reduction in number of writhing. It was observed that petroleum ether and ethyl acetate more significant analgesic activity, when compare to methanol. The effect of *M. dioica* fruit seed extracts showed dose dependent reduction in the number of writhing as compared to control drug which was highly significant (P<0.01) (Table 4).

3.3.2. Hot plate method in mice

M. dioica fruit seed extracts pretreatment increased the response latency in the hot plate test which was significant. The control drug increased the response latencies at various time intervals. The effect of *M. dioica* fruit seed extracts were dose as well as time dependent. Amongst all the doses used, petroleum ether extract was most effective at 50 and 100mg/kg at 90 and 120 minutes, as well as methanol extract at 50 mg/kg at 60 and 90 minutes comparable as the control drug which was highly significant (P<0.05, P<0.01) (Table 5).

4. Discussion

Certain flavonoids are reported to exhibit anti-inflammatory, antioxidant and hepatoprotective activities [19]. Phytochemical analysis of the methanol extract shows the presence of carbohydrates, saponins, triterpenoids and flavonoids. Thus we can speculate that these constituents might be responsible for antiallergic activity. Literature shows that flavonoid is having antianaphylactic and antiasthmatic activity. So antiallergic activity showed by *M. dioica* fruit seed might be induced by these chemical moieties. After parenteral administration of milk there is increase in total leukocyte count (TLC) and this stressful condition can be normalized by administration of an antistress or adaptogenic drug. Eosinophilia is an abnormal increase in peripheral eosinophil count to more than 4 % of total leukocytes. In the late phase, especially in the development of allergic asthma, eosinophils play role as an inflammatory cell [20, 21]. It was also demonstrated that parental administration of milk produces a marked and significant increase in the leukocytes or eosinophils count after 24hr of its administration [8]. There is an insufficient quantity of data about the pharmacological activities of *M. dioica* fruit seed, which prompted us to carry out its extracts pharmacological evaluation to verify the

medicinal properties as well as responsible phytochemical constituents.

Conflict of interest statement

We declare that we have no conflict of interest.

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