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Analgesic Activity of *Abelmoschus manihot* Extracts

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Abstract: The natural products served as important sources of medicines now a day increasing, as they possess the therapeutic activity. Therefore, the present study was carried out to evaluate the analgesic activity of the petroleum ether and methanol extract of *Abelmoschus manihot* (Malvaceae) leaves using hot plate and tail immersion model. The air-dried, powdered leaves (1000 g) were extracted over Soxhlet with petroleum ether and methanol. The crude dried petroleum ether (10 g) and methanol (25 g) extracts was prepared at the doses of 100, 200 and 400 mg kg^{-1} and evaluated for analgesic activity using the hot plate and tail immersion test. The results obtained indicate that the extracts possessed significant ($p<0.05$, $p<0.01$) analgesic activity, which was found to be dose-dependent. A significant inhibition in pain threshold in hot-plate test was exhibited. However, in flick test, highest analgesic activity was observed only with 400 mg kg^{-1} dose as compared with the standard drug. This study showed that the petroleum ether and methanol extracts of *Abelmoschus manihot* leaves possess potential pharmacological active constituents responsible for inhibition of the analgesic effect.

Key words: *Abelmoschus manihot*, analgesic activity hot plate tail immersion swiss albino mice

INTRODUCTION

There is an increasing trend of herbal formulation as they have lesser side effects and are easy to access as well as in some cases these are the only available treatment against chronic diseases (Karim *et al*., 2011). *Abelmoschus manihot* (Linn.) belongs to family Malvaceae is a large annual erect hairy plant, 1.2-1.8 m. high, stems with small scattered prickles. Leaves 15-18 cm, deeply palmately lobed, stipules are 1 cm long, linear-lanceolate with stiff bristles on the margins. Flowers yellow with a purple centre, capsules oblong, pointed, hispid, seeds black (Pullaiah, 2002). The mucilage contains polysaccharides and proteins (Kiritikar and Basu, 1994). The flower contains quercetin-3-robinoside, quercetin-3'-glucoside, hyperin, myrecetin and anthocyanins (Lai *et al*., 2006). The different chromatographic methods have been developed on the flavones present in the plant (Liang *et al*., 2007; Lai *et al*., 2009; Yi *et al*., 2008). The flowers are used in the treatment of chronic bronchitis and toothache. The ethanol extract of flower was screened for antiviral activity and it was observed that the hyperoside shown significant anti HBV activity (Lin-Lin *et al*., 2007). The phytoconstituents obtained from the plant possesses different pharmacological activities (Liu *et al*., 2009; Wen and Chang, 2007; Xin-Ping *et al*., 2006). The leaves were tested on bone loss in ovariectomised rats and it was observed that it was able to prevent the ovariectomy-induced femoral osteopenia (Puel *et al*., 2005).

The phytochemical analysis showed the presence of steroids, triterpenoids and flavonoids in petroleum ether and methanol extract, respectively which possesses analgesic (Gupta *et al*., 2008) antioxidant and anti-inflammatory activity (Oyedapo *et al*., 2008; Raj *et al*., 2006). Therefore, in this study the analgesic activity evaluated of the leaves of *Abelmoschus manihot*.

MATERIALS AND METHODS

Plant material: The leaves of *Abelmoschus manihot* were collected in August 2010 from Toranmal Hills of Maharashtra and authentication of the plant specimen was established by Dr. D.A. Patil was deposited in our departmental herbarium for future reference (Patil, 2003).

Preparation of extracts: The leaves were chopped into small pieces air-dried and powdered. The powdered plant material was successively soxhlet extracted with petroleum ether and methanol at room temperature for 48 h.

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Standard drugs/chemicals solutions: Pentazocine (Neon) (10 mg kg^{-1}), used for the purpose of comparison, was prepared by dissolving, in acacia suspension. Carrageenan (λ4) (C3889-5G) was obtained from Sigma Aldrich, USA.

Pharmacological studies

Animals: Swiss albino mice (25-30 g; 7-10 weeks old) were, obtained from RC Patel Institute of Pharmaceutical Education and Research, Shirpur and Government Veterinary College, Mahu, MP (India). The animals were housed in Animal house of R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India in polycarbonate cages, in a room maintained, under controlled room temperature 22±2°C, relative humidity 60-70% and provided with food and water *ad libitum*. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India. The animals were deprived of food for 24 h before experimentation but allowed free access to water throughout. All studies were carried out by using six animals in one group for anti-inflammatory activity.

Acute toxicity test: A cute toxicity test was performed according to OECD (2006) guidelines. Animals were weighed and marked, a single high dose, 2000 mg kg^{-1} of petroleum ether extract and methanol extract as recommended by the OECD guidelines was administered to the first animal. After a single administration, a sign of toxicity and behavior was observed each hour up to the 24 h. If this animal was dying, then one lesser dose of that dose was administered to the next animal. Same procedure was followed for that animal. If the animal survives, then the same dose was given to the next 5 animals. All the animals were observed for the signs of toxicity and mortality for up to the 14 days.

Additional observations like changes in skin, eyes and mucous membranes and also respiratory circulatory, autonomic and Central Nervous system and behavior pattern. Attention was also given to observed precipitation of tremors and convulsions.

Hot plate method: The Swiss albino mice (25-30 g) of either sex were divided into eight groups containing six animals in each. A control group received normal saline solution, while three groups received petroleum ether extract at doses 100, 200 and 400 mg kg^{-1} p.o, respectively. The remaining three groups received methanol extract at doses 100, 200 and 400 mg kg^{-1} p.o. Pentazocine (10 mg kg^{-1}) was administered to eighth group. The temperature of hot plate was maintained at 55±0.5°C. The rats were placed individually on hot plate and time between placement and licking of paws, shaking or jumping off the surface was recorded by using Eddys hot plate apparatus (Spacelab). As a response latency rat with baseline latencies of less than 5 sec or more than 15 sec were eliminated from the study and cut off latency time was set at 15 sec to avoid tissue damage. After determination of base line response latencies, hot plate latencies were re determined at 0, 30, 60, 90 and 180 min after drug administration (Oloyede *et al*., 2008).

Tail Immersion method: The Swiss albino mice (25-30 g) of either sex were divided into eight groups containing six animals in each. The rats were fasted for 12 h prior to induction of analgesia. The control group received normal saline solution, while three groups received petroleum ether extract at doses 100, 200 and 400 mg kg^{-1} p.o., respectively. The remaining three groups received methanol extract at doses 100, 200 and 400 mg kg^{-1} p.o. Pentazocin (10 mg kg^{-1}) was administered to eighth group. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water of exactly 55°C. Within a few seconds the rat reacted by withdrawing the tail. The reaction time was recorded by a stopwatch. After each determination the tail was carefully dried by a cloth. The reaction time was determined before and periodically after oral administration of the test substance, e.g., after 0, 30, 60, 90 and 180 min. The cut off time of the immersion was kept 15 sec. The withdrawal time of untreated animals was between 1 and 5.5 s. A withdrawal time of more than 6 sec therefore is regarded as a positive response.

Statistical analysis: The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnett's multiple comparisons using graph pad in stat 3 Demo and obtained in the study were compared with the vehicle control group. The p values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Extraction: The percentage of yield of the petroleum ether and methanol extracts was 0.10 and 0.25% , respectively.

Acute toxicity test: The *Abelmoschus manihot* leaf extracts did not produce any mortality even at the dose of 2000 mg kg^{-1}, p.o., thus found to be non-toxic. Therefore, doses (100, 200, 400 mg kg^{-1}, p.o.) of

Table 1: The analgesic activity of petroleum ether and methanol extract of *Abelmoschus manihot* using the Hot plate method

		Reaction time in sec				
Treatment	Dose (mg kg^{-1})	0 min	30 min	60 min	90 min	180 min
Control		3.86±0.38	4.43±0.39	4.58±0.69	4.85±0.24*	4.94±0.24*
Petroleum ether extract	100	6.44±0.47*	8.48±0.23*	11.11±0.25*	13.36±0.18*	13.90±0.12*
	200	6.48±0.55*	8.55±0.36*	11.51±0.27*	13.41±0.56*	14.00±0.15*
	400	6.98±0.29*	8.60±0.27*	11.71±0.38*	13.60±0.48*	14.10±0.21*
Methanol extract	100	6.43±0.47*	8.48±0.23*	11.11±0.25*	13.36±0.18*	13.90±0.12*
	200	6.75±0.55*	8.38±0.41*	10.98±0.35*	12.28±0.27*	14.10±0.15*
	400	6.98±0.29*	8.60±0.27*	11.71±0.38*	13.60±0.49*	14.10±0.21*
Pentazocin	10	6.80±0.64**	11.40±0.51**	14.52±0.78**	14.90±0.68**	17.20±0.1**

Values represent Mean±SEM, n = 6. One way ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01 compare with control group

Table 2: The analgesic activity of Petroleum ether and methanol extract of *Abelmoschus manihot* Assessed using Tail Immersion method

		Mean reaction time in sec				
Treatment	Dose (mg kg^{-1})	0 min	30 min	60 min	90 min	180 min
Control		1.86±0.22	1.95±0.11	2.02±0.32	2.36±0.21	2.98±0.11
Petroleum ether extract	100	2.61±0.16**	3.91±0.11**	4.05±0.11**	4.92±0.11**	4.98±0.26**
	200	3.61±0.13**	4.02±0.12**	4.98±0.11**	5.02±0.23**	5.00±0.12**
	400	4.83±0.12**	5.03±0.13**	5.30±0.14**	6.02±0.22**	6.98±0.21**
Methanol extract	100	2.61±0.08**	3.91±0.12**	4.05±0.12**	4.92±0.35**	4.98±0.24**
	200	3.66±0.19**	4.05±0.20**	4.96±0.21**	5.06±0.32**	5.38±0.25**
	400	4.96±0.21**	5.02±0.11**	5.96±0.11**	6.02±0.12**	7.05±0.11**
Pentazocin	10	8.23±0.15**	8.95±0.14**	9.22±0.13**	10.40±0.12**	11.06±0.11**

Values represent Mean±SEM, n = 6. One way ANOVA followed by Dunnett's multiple comparison test **p<0.01 compare with control group

A. manihot three was selected for further pharmacological studies (Table 1-2).

Hot plate method: The petroleum ether and methanol extracts at the doses of 100, 200 and 400 mg kg^{-1} p.o. showed significant ($p<0.05$, $p<0.01$) and dose dependent increase in the reaction time at 180 min (Table 1). The petroleum ether and methanol extract in the dose of 400 mg kg^{-1} showed maximum of 14.1±0.21 and minimum of 6.98±0.29 reaction time which showed significant threshold in pain , respectively while the group treated with standard drug Pentazocin (10 mg kg^{-1}) showed significant ($p<0.01$) increase in the reaction time as compared to control.

Tail immersion method: The petroleum ether and methanol extracts at the doses of 100, 200 and 400 mg kg^{-1} p.o. showed significant ($p<0.05$, $p<0.01$) and dose dependent increase in the reaction time at 180 min (Table 2). The flick test showed the petroleum ether extract showed 6.98±0.21 and 4.83±0.12 reaction time while methanol extract 400 mg kg^{-1} showed 7.05±0.11 and 4.96±0.21 reaction time, exhibited highest analgesic activity, while the group treated with standard drug Pentazocin (10 mg kg^{-1}) showed significant ($p<0.01$) increase in the reaction time as compared to control.

The present study indicated that *A. manihot* has the pharmacological potential as an analgesic agent when tested on various animal models. The ability to inhibit/reverse the former and latter tests could also be associated with the extract's potential to inhibit the induced analgesia (Ballou *et al*., 2000). Although the present study did not aim at isolation and identification of bioactive compounds the phytochemical screening of petroleum ether and methanol extract demonstrated the presence of flavonoids, steroids, triterpenoids, which are suggested to act synergistically to exert the observed pharmacological activity (Maj and Rogoz, 2000). The presence of steroids and flavonoids in petroleum ether and methanol extract could possibly lead to the observed activity. The analgesic activity of petroleum ether and methanol extract could also be linked to the mechanism of action either on central nervous system or peripheral nervous system. Interestingly, compounds like flavonoids (Kim *et al*., 2004) and steroids, triterpenes in part, have been shown to possess anti-inflammatory, analgesic activity and the claim made by Attaway and Zaborsky (1993). Based on the classes of compounds detected in petroleum ether and methanol extract, several mechanisms of action could be used to explain the observed activities of the extract. Flavonoids are potent inhibitors of nitric oxide synthase type 2 that are involved in the synthesis of NO (Olszanecki *et al*., 2002). The ability of steroids and flavonoids to induce vasodilatation and the importance of vasodilation in the antinociceptive and anti-inflammatory mechanisms have also been reported and are worth mentioning (Naseri *et al*., 2005). To demonstrate whether petroleum ether and methanol extract is producing analgesic activity in this model by acting on Pentazocin,

further the effect of petroleum ether and methanol extract was studied on Pentazocin induced analgesia.

In hot plate and tail immersion analgesic models petroleum ether and methanol extract and reference drug Pentazocin significantly increased the reaction time at 180 min. In the late phases of this model petroleum ether, methanol extract, Pentazocin showed analgesic activity. The results of present study support the ethno-medical application of *A. manihot* in the treatment of analgesic diseases. Further experimentation is needed in order to understand the precise mechanism of action in analgesic activities by the extracts.

CONCLUSION

The present study demonstrated that the petroleum ether and methanol extracts possessed analgesic activity. Thus, the present study confirmed the folklore use of *A. manihot* fruit for the treatment of various ailments and the plant's potential pharmacological activities merit further investigation.

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