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EXPERIMENTAL EVALUATION OF ANALGESIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF MANSOA ALLIACEA (LAM.) LEAF

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ABSTRACT

Mansoa alliacea or Garlic vine plants are not only an ornamental. Garlic vine has many properties like anti-inflammatory, ant-arthritic, anti-oxidant, anti-cancer therefore in the present study *Mansoa alliacea* leaves hydroalcoholic extract preparation was evaluated for its analgesic efficacy by making use of different central and peripheral pain models in mice. **Material and Method:** The analgesic efficacy of hydroalcoholic extract was assessed by employing different pain models such as i) Acetic acid induced writhing reflex method as peripheral analgesic model ii) Tail immersion model iii)Tail flick model for central analgesia. The percentage inhibition of writhes and prolongation of reaction time were the parameters of evaluation. The results obtained were analysed by ANOVA and Student's unpaired "t"- test. **Result:** Hydroalcoholic extract of *M. alliacea* (Lam.) leaf (100 & 200 mg/kg) reduced writhing episodes significantly in 3% of acetic acid induced writhing in mice as compared to control indicating it's analgesic effect. The highest percentage inhibition of pain was seen with 200 mg/kg of hydroalcoholic extract of *Mansoa alliacea* (Lam.) leaf. Tail immersion model the standard drug morphine (5 mg/kg) showed 18.90 protection of analgesia while the *M. alliacea* leaf (100 & 200 mg/kg) produced 7.78 & 8.80 protection of analgesia respectively which is significant. In tail flick method

hydroalcoholic extract of the *M. alliacea* leaf showed analgesic activity as there was significant increase in Tail flick latency time from 0 to 180 min. The maximum analgesic effect was shown at higher dose (200mg/kg, p.o.) which was comparable to that of diclofenac. **Conclusions:** Hydroalcoholic extract of *Mansoa alliacea* (Lam.) leaf was found to be effective in all three models of experimental pain. However it is less potent than standard analgesic drugs and could be employed safely in higher doses.

KEYWORDS: Analgesic, hydroalcoholic extract, pain models.

INTRODUCTION

Mansoa alliacea Lam. (Family Bignoniacea) is a native plant from Amazonian basin. This plant is mainly found in Southern America but it is alsofound tropical rain forest region in India.There are total 11 species. *Mansoa alliacea* have several vernacular names like Fake garlic in English[1], Wild garlic in English[1], Ajos sacha in Span[1], Garlic Vine in English[2], Other -Bejuco de ajo, Mata de ajo; Garlic vine[2], Bejuco De Ajo in Spanish[2], Lasun Vel, Lasnya in Marathi[3], Lahan Bel in Hindi[3] *Mansoa alliacea* is a native Amazonian plant belonging to the family of Bignoniaceae, its scientific name is *Mansoa alliacea* (Lam.) A. Gentry but has been classified with several synonyms.[4] The name ajo sacha means 'false garlic', due to the characteristic garlic smell molecules present into the leaves.[5] Genrally leaves are used in the preparation of infusion or decoction. Roots are used in preparation of cold maceration and tincture and generally taken as a whole body tonic.[6,7]

AIMS AND OBJECTIVES

The present investigation deals with the pharmacological evaluation of hydroalcoholic extract of *M. alliacea* (Lam.) leaf.

MATERIAL AND METHODS

Collection of the plant

Mansoa alliacea (Lam.) leaves was collected from Herbal Garden of Govt. Ayurved College Raipur (C.G.) in India.

Authentication of the Drug

Taxonomic identification of collected material was done in the Raw Material Herbarium & Museum, Delhi (RHMD), National Institute of Science Communication and Information Resources (CSIR-NISCAIR).

PHARMACOLOGICAL STUDY

Materials and Methods

Swiss albino rats of either sex (220-250g) were obtained from Animal house of the Indira Gandhi Institute of Pharmaceutical Sciences, IRC village, Nayapalli, Bhubaneswar, Odisha. The animals were kept in polypropylene cages at 25 ± 2° C with relative humidity 45-55% under 12 hr light and 12 hr dark cycle. They were fed with standard laboratory animal fed and tap water ad libitum. All the pharmacological and toxicological experimental protocols were approved by Institutional Animals Ethics Committee [IAEC] for the purpose of Control and Supervision on Experimentation on Animals [CPCSEA].vide sanction Regd. No. 1025 C/07/CPCSEA, dt. 24.01. 2007.

Statistics

The results are expressed as mean ± SEM. The statistical difference between control and treated groups were tested by Student's 't' test. In all cases, a difference was considered significance when $P<0.001$.

Hydroalcoholic extraction of plant material[8]

The coarse powder (500 gms) of leaf of *Mansoa alliacea* (Lam.) has been used for extraction process following Maceration method. Coarsely ground powder of the *Mansoa alliacea* (Lam.) leaf has been placed in one large glass container and approximately 1550 ml of 80% Ethanol has been added to it for maceration in order to get a hydro-alcoholic extract. The glass container shall be closed with a glass lid to prevent evaporation of the menstruum and this system has been allowed to stand for 7 days with occasional stirring. The liquid i.e. the menstruum has been then strained and the solid residue, called marc, has been pressed to recover as much occluded solution as possible. The strained and expressed liquid thus obtained will be mixed and clarified by filtration. The filtration has been carried out in a beaker using a Whatman's filter paper no 1. China dishes has been used for evaporation of the menstruum. These china dishes containing the menstruum has been placed on a water bath. After evaporation of the menstruum of the hydro-alcoholic extract has been collected. These extract has been stored in a dark colored pre-sterilized airtight container. It has been then stored in a refrigerator at 4^0C in a dark colored pre-sterilized airtight container until its further use.

Acute toxicity study[8]

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development-423 [OECD-423] guidelines (acute toxic class method). Albino rats (n=6) of either sex was selected by random sampling technique. The animals were kept fasting for overnight, had access only to water. The hydroalcoholic extract of *M. alliacea* leaf was administered orally at the initial dose 5 mg/kg body weight by intra gastric tube and observed for 14 days. The animals are observed individually after dosing once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. Since there was no mortality with 5 mg/kg for 14 days, the procedure was repeated for next higher doses such as 50, 200 and 2000 mg/kg.

Evaluation of Analgesic activity[8]

Pain is not easily or satisfactorily defined and therefore is often interpreted as a suffering that results from the perception of painful stimuli. It's a common symptom and it indicates that something is wrong in the body and may give a clue to the nature of disease. Hence, "pain is a specific sensation with its own peripheral and central mechanisms independent of other five senses." Pain itself is not a disease; it is by far the most common medical complaint. It is usually perceived as an indication of ill health and most diseases have a component of pain. The control of pain is one of the most important uses to which drugs are put. Pain can be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain. Drugs, which alter the pain sensitivity or remove pain, are called as painkiller or analgesic.

I. Tail immersion model

Animals

Wistar albino female rats.

MATERIALS

Hydroalcoholic extract of *M. alliacea* leaf in 1% v/v Tween 80 emulsion and Morphine (MO) injection.

Experimental protocol

Rats (six per group) were randomly divided in to four groups. Group I animals received only 1% v/v Tween 80 solution (10 ml/kg, p.o.). Group II & III animals received hydroalcoholic extract.of *M. alliacea* leaf 100 and 200 mg/kg, p.o., in 1% v/v Tween 80 emulsion

respectively. Group IV animals received Morphine (5 mg/kg, s.c.). The animals were screened for the sensitivity test by immersing 3 cm of the tail of the rat gently in hot water maintained at 55 ± 0.5 °C. Within a few sec, the rats reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. Each animal served as its own control and two readings were obtained for the control at 0 and 10 min interval. The average of the two values was taken as the initial reaction time. After 30 min, tail withdrawal time of each group animals was noted and the % protection of analgesia was calculated by using the formula C-T/C x 100 where 'C' represents the tail withdrawal (in sec) of control and 'T' to that of treated groups.

II. Tail flick method

Animals

Wistar albino female rats.

MATERIALS

Hydroalcoholic extract of *M. alliacea* leaf in 0.2 ml of 2% v/v Caboxy methyl cellulose suspension orally and Diclofenac sodium suspended in 2% CMC intraperitoneally.

Experimental protocol

The rats were randomly assigned to four groups of six animals each. The first group served as negative control receiving 2% w/v caboxy methyl cellulose suspension orally (0.2 ml/kg). The second and third groups served as test group and were given hydroalcoholic extract of the *M. alliacea* leaf was given at a dose of 100 mg/kg & 200 mg/kg suspended in 2% CMC orally respectively. The last group served as control group was given Diclofenac sodium suspended in 2% CMC intraperitoneally.

Antinociceptive (analgesic) activity of the extract was evaluated by the tail-flick method described. About 5 cm from the distal end of the tail of each rat was immersed in warm water maintained at 50 ± 5°C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 30, 60, 120, and 180 min after the administration of the treatments. The maximum reaction time was fixed at 10 sec to prevent any tail tissue injury. If the reading exceeds 10 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

MPA=Reaction time for treatment−reaction time for saline/10 sec−reaction time forsaline×100

III. Acetic acid induced writhing reflex method

Materials

Hydroalcoholic extract of *M. alliacea* leaf in 1% v/v Tween 80 emulsion and Ibuprofen (100 mg/kg, p.o.) in 1% w/v SCMC suspension were used for the study. 1% v/v Tween 80 (10 ml/kg, p.o.) solution was used as vehicle control.

Animals

Wistar albino mice (25-30g) of either sex.

Experimental protocol

Mice of either sex were randomly divided into four groups, each group containing six animals. Group I animals received only 1% v/v Tween 80 solution (10 ml/kg, p.o.). Group II & III animals received hydroalcoholic extract of *M. alliacea* leaf 100 and 200 mg/kg, p.o., in 1% v/v Tween 80 emulsion respectively. Group IV animals received Ibuprofen (100 mg/kg, p.o. in 1% w/v SCMC), All the animals received intra peritoneal (i.p.) injection of 3% v/v of acetic acid (1 ml/100g) 30 min after the administration of drugs *M. alliacea* leaf (100 & 200 mg/kg) in 1% v/v Tween 80 emulsion and Ibuprofen (100 mg/kg). Ibuprofen was used as the standard drug. The number of writhings produced by each animal was observed individually under a glass jar for a period of 20 min and the same was counted. The % protection of analgesic activity was calculated by using the formula C-T/C x 100, where C is the number of writhings in the control group and T is the number of writhings in the treated group.

RESULTS AND DISCUSSION

Acute toxicity studies

Administration of 2000 mg/kg, p.o. of the hydroalcoholic extract of *M. alliacea* leaf did not produce any behavioral abnormalities and mortality and was considered as safe (OECD-423 guideline unclassified).Acute toxicity test of hydroalcoholic extract of *M. alliacea* shown in Table No.1.

Table No. 1: Acute toxicity test of hydroalcoholic extract of *M. alliacea* leaf.

S. No.	Extracts 2000 mg/kg, p.o.	No. of animals dead/survived
1.	Hydroalcoholic extract	0/6

Pharmacological assessment

Analgesic activity

Acetic acid induced writhing response in mice

Analgesic activity of hydroalcoholic extract of *M. alliacea* leaf and Ibuprofen by acetic acid induced writhing reflex method is shown in Table No. 2.

Table No. 2: Analgesic activity of hydroalcoholic extract of *M. alliacea* leaf and Ibuprofen.

Treatment	Dose	Mean Writhings	% Protection
1% v/v Tween 80	10ml/kg	49.66±6.8	-
Hydroalcoholic extract of *M. alliacea* leaf	100mg/kg	32.66±3.48*	46.22
	200mg/kg	27.82±1.28**	56.28
Ibuprofen	100 mg/kg	23.50±2.42***	63.08

All values represented as Mean±SEM and values are overall significant. One way ANOVA; n=6 in each group in seconds. * P<0.05; **P<0.001; ***P<0.0001.

Hydroalcoholic extract of *M. alliacea* leaf (100 & 200 mg/kg) has significantly reduced the number of writhe induced by acetic acid at dose of 10 ml/kg. The number of writhe in the acetic acid vehicle control group was found to be 49.66±6.8. This reduction was dose related. *M. alliacea* leaf (100 & 200 mg/kg) produced 46.22 and 56.28% protection (P<0.05 & P<0.001) respectively. Ibuprofen (100 mg/kg) appears to be more effective in reducing the number of writhe, it has significantly (P<0.0001) reduced the number of writhe by 63.08%.

Tail immersion model

Analgesic activity of *M. alliacea* leaf and Morphine by tail immersion model are shown in Table No. 3.

Table No. 3: Analgesic activity of *M. alliacea* leaf and Morphine.

Treatment	Dose	Reaction time in seconds	
		Initial (Mean ± SEM)	60 Min (Mean ± SEM)
1% v/v Tween 80	10ml/kg	1.80±0.14	-
Hydroalcoholic extract of *M. alliacea* leaf	100mg/kg	2.64±0.032	7.78±0.012*
	200mg/kg	3.22±0.046	8.80±0.184**
Morphine	5 mg/kg	3.82±0.040	18.90±0.030***

All values represented as Mean±SEM and values are overall significant. One way ANOVA; n=6 in each group in seconds. * P<0.05; **P<0.001; ***P<0.0001.

In tail immersion model, the standard drug morphine (5 mg/kg) showed 18.90 protection (P<0.0001) of analgesia while the *M. alliacea* leaf (100 & 200 mg/kg) produced 7.78 & 8.80 protection of analgesia respectively which is significant.

Tail flick method: Analgesic activity of *M. alliacea* leaf and Diclofenac by tail flick method are shown in Table No. 4.

Table No. 4: Analgesic activity of *M. alliacea* leaf and Diclofenac.

Groups	Dose	Reaction time in seconds at time minutes(ms) Mean ±SEM				
		0ms	30ms	60ms	120ms	180ms
Group I	0.2ml	1.96±0.22	2.42±0.02	2.42±0.08	2.36±0.06	2.34±0.06
Group II	100mg/kg	2.06±0.06	3.62±0.01	6.86±0.04	7.18±0.02	6.14±0.08
Group III	200mg/kg	2.14±0.02	5.86±0.02	10.12±0.01	11.20±0.08	11.8±0.08
Group IV	1mg/kg	2.08±0.02	6.76±0.02	13.42±0.04	13.16±0.06	13.2±0.02

All values represented as Mean±SEM and values are overall significant. One way ANOVA; n=6 in each group in seconds. * P<0.05; **P<0.001; ***P<0.0001.

In this method hydroalcoholic extract of the *M. alliacea* leaf showed analgesic activity as there was significant increase in Tail flick latency time from 0 to 180 min. The hydroalcoholic extract of *M. alliacea* significantly attenuated the spinal pain sensation against conduction heat in mice. Moreover, this ameliorative effect of tail withdrawal response was observed in a dose dependent manner. The maximum analgesic effect was shown at higher dose (200mg/kg, p.o.) which was comparable to that of diclofenac (1mg/kg, p.o.). At the dose of 100mg/kg; it showed highly significant activity $p < 0.05$ at 60, 120, where as the most significant effect p<0.001 was observed at the dose of 200mg/kg at 60 and 120min. Diclofenac sodium used as standard analgesics exerted a highly significant effect (p<0.0001) at 60min.

CONCLUSION

In the present investigation it could be concluded that the hydroalcoholic extract of *Mansoa alliacea* (Lam.) at tested doses exhibited the pharmacological activities. In analgesic activity, the *Mansoa alliacea* (Lam.) exhibits the analgesic activity, in which its mechanism of action is peripherally and centrally mediated levels which is shown in a Table no. 2, 3 and 4. Based on these results, it is clearly that the hydroalcoholic extract of *Mansoa alliacea* (Lam.) leaf possesses analgesic activity in experimental animal models which support the traditional uses of *M. alliacea* (Lam.) leaf in pain and inflammation of arthritis and rheumatism.

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