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Research Article

PHARMACOLOGICAL SCREENING OF ISOLATED COMPOUND FROM *MADHUKA LONGIFOLIA* SEEDS GIVES SIGNIFICANT ANALGESIC EFFECT

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ABSTRACT
The study was carried out to assess the analgesic effect of aqueous and ethanolic extracts of isolated compound from *M.longifolia* seeds in rats and mice model. All three animal groups were administered the aq. and alc.ext of *M.longifolia* at a dose of 4 mg to 64 mg/kg body weight. The standard drug diclofenac 5 mg/kg b.w is used in three screening method. The paw licking time, tail withdrawal time and chemical writhings in mice both aq. and alc. extracts of *M.longifolia* prevents significant dose dependent anti-nociceptive effect. Diclofenac 5 mg/kg failed to alter significantly the antinociceptive effect of 16 to 32 mg of both extracts or the effect on chemical assay.
KEY WORDS: Analgesic effect, *Madhuca longifolia* seeds, Alcoholic extract, Aqueous extract.

INTRODUCTION

Madhuca longifolia, commonly known as mahwa or mahua, is an Indian tropical tree found largely in the central and north Indian plains and forests. It is a fast growing tree that grows to approximately 20 meters in height, possesses evergreen or semi-evergreen foliage, and belongs to the family Sapotaceae. It is adapted to arid environoments, being a prominent tree in tropical mixed deciduous forests in India in the states of Jharkhand, Uttar Pradesh, Bihar, Madhya Pradesh, Kerala, Gujarat and Orissa.The tree is considered a boon by the Tribals who are forest dwellers and keenly conserve this tree. However, conservation of this tree has been marginalized, as it is not favoured by non-tribals.The leaves of *Madhuca indica* (= *M. longifolia*) are fed on by the moth Antheraea paphia which produces tassar silk (Tussah), a form of wild silk of commercial importance in India.Mowrah seed (*Madhuca latifolia*) meal[†] contains high levels of saponin (7%) making it unsuitable for incorporation in animal feedstuff formulations. The saponin from mahwa seed meal was isolated and purified by paper chromatography. This was used for *in vitro* tests as well as pharmacological and acute toxicity studies for a better understanding of its properties and toxicity. The oral, intraperitoneal and intravenous LD_{50} of mahwa saponin in mice are 1 g, 15–20 mg and 15 mg/kg body weight respectively. Processing of the meal to remove or inactivate the saponin will be essential prior to its incorporation in animal feeds.

Previous phytochemical studies on *Madhuca longifolia* include characterization of sapogenins, carbohydrate-s,triterpenoids, steroids, saponin, flavonoids and glycosides. Triterpen glycosides, madlongisides A-D, were isolated from the seeds of *Madhuca longifolia* and the structures were elucidated on the basis of extensive NMR experiments.

MATERIALS AND METHODS

Preparation of Extracts

The air-dried seeds (2.5 kg) of *M. longifolia* were prepared with water or 98% ethyl alcohol in Soxhlet apparatus were defatted with petroleum ether. The extract was evaporated to dryness in vacuo. Albino rats (120 to 150 g) or albino mice (20 to 30 g) of either sex were taken for the study. The anti - nociceptive activity was evaluated by administering the varying doses of extracts intramuscularly for 3 days and last dose was administered 30 mints. before the experiment. The doses and time were selected based on preliminary screening method.

Acute toxicity and selection of doses

The acute toxicity studies were carried out in adult albino rats 120 – 150 (g), by up and down method as per OECD 425 guidelines. Overnight fasted animals received test drug 50 to 170 mg per kg body weight orally. Then the animals were observed continuously once in half an hour for the next four hours and then after 24 hours for general behavioral, neurologic and autonomic profiles and to find out mortality. The extract was found safe to up to a dose of 170 mg /kg body weight.

Anti – nociceptive effect

It was carried out by using tail-flick or hot plate method in rats and by acetic acid induced abdominal constrictions assay procedure in mice.

Procedure

In the tail – flick method distal part of rat's tail was immersed in hot water at 55.0 ± 1.0° C. The time taken by the rats to withdraw the tail was noted as reaction time .In the hot plate method the rats were placed on the hot plate at 55.0 ± 1.0° C and the time taken to lick the hind paw (reaction time) was noted. The animals having reaction time within 30 sec. were include in the study. The chemical writhing method involves 10 mg/kg, i.p. injection of freshly prepared 0.6% acetic acid. The number of abdominal constrictions in the following 10 mints. were noted

Significant reduction either in the reaction time abdominal constrictions compared with vehicle treated animals was considered as anti – nociceptive response. The results of the thermal assay have been expressed as area under the curve calculated by "Pharmabit Software" while those of chemical assay as the number itself.

To elucidate the opioid pathways in the anti – nociceptive effect of *M. longifolia*, diclofenac 25 mg/kg body weight was administered 30 mins. before the last injection of the extract.

Statistical analysis

Data were statistically evaluated by use of one-way ANOVA, followed by Dunnet`s "t" test. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Graded doses of the extracts (aqueous and methanolic) of *M.longifolia* seeds varying from 4.0 mg to 64.0 mg/kg increased significantly in dose dependent manner the paw licking time (Table 1) as well as the time taken for withdrawal of tail (Table 2) in rats shown as area under the curve. But 4.0-8.0 mg/kg i.m aqueous and methanolic extracts from seeds of *M.longifolia* in mice significantly decreased (Table 3). Diclofenac 5 mg/kg failed to alter significantly the antinociceptive effect of 16 or 32 mg of methanolic or aqueous extracts of *M.longifolia* or their effect on chemical assay.

Aqueous and methanolic extracts of *M.longifolia* presents significant dose dependent antinociceptive effect as measured by tail flicks, hot plate methods in rats and chemically induced abdominal constrictions in mice. To conclude the analgesic effect of both extracts obtain from the seeds of *M.longifolia* . It was not altered by diclofenac in both rats and mice indicating their nociceptive effect is not mediated through opioid pathway.

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Table 1:- Showing the effect on paw licking time (as area under the curve) of diclofenac sodium on analgesic effect of aqueous and alcoholic extracts of *M. longifolia*.

Extract & dose (mg/kg, i.m)	Area under curve (in cm^2 ±S.E.)	Area under curve (in cm^2 ±S.E.)
	Normal	Diclofenac
Saline	48.22 ± 0.78	53.66 ± 0.26*
Aq. Extract of M.longifolia	49.36 ± 0.05	—
4	55.88 ± 0.72*	—
8	56.18 ± 0.68**	73.66 ± 0.31** ^{xx}
16	87.44 ± 0.70**	91.20 ± 0.18** ^{xx}
32	83.05 ± 0.73**	—
64		
Saline	34.30 ± 0.32	55.22 ± 0.16** ^{xx}
Alc.Extract of M.longifolia		—
4	46.11 ± 0.52	—
8	59.33 ± 0.05**	77.44 ± 0.13** ^{xx}
16	60.25± 0.65**	95.05 ± 0.22** ^{xx}
32	70.66 ± 0.72**	
64	74.68 ± 0.78 **	—

n =6 rats in each group.*P<0.05, **P<0.01 significantly different as compared to control group. ^{xx} P <0.01 as compared to diclofenac control group.

Table 2:- Showing the effect on tail withdrawal time (as area under the curve) of diclofenac sodium on analgesic effect of aqueous and alcoholic extracts of *M. longifolia*.

Extract & dose (mg/kg, i.m)	Area under curve (in cm^2 ±S.E.)	Area under curve (in cm^2 ±S.E.)
	Normal	Diclofenac
Saline	15.21 ± 0.13	32.22 ± 0.20*
Aq. Extract of M.longifolia		
4	17.33 ± 0.41	—
8	23.05 ± 0.28*	—
16	27.11 ± 0.30**	46.90 ± 0.22** ^{xx}
32	36.67 ± 0.52**	64.66 ± 0.18** ^{xx}
64	76.06 ± 0.73**	—
Saline	10.21 ± 0.12	32.05 ± 0.16** ^{xx}
Alc.Extract of M.longifolia		
4	16.32 ± 0.12	—
8	19.45 ± 0.15**	—
16	32.06 ± 0.42**	41.05 ± 0.13** ^{xx}
32	39.98 ± 0.42**	61.25 ± 0.22** ^{xx}
64	48.72 ± 0.33 **	—

n =6 rats in each group.*P<0.05, **P<0.01 significantly different as compared to control group. ^{xx} P <0.01 as compared to diclofenac control group.

Table 3:- Effect on diclofenac sodium and its combination with aqueous or alcoholic extracts from of *M. longifolia* seeds on chemical writhings in mice.

Group	**No of writhing (Mean ± S.E) in 10 min**
Normal control	16.11 ± 0.91
Diclofenac (5 mg)	14.17 ± 0.66
Aq.extract (4 mg)	9.68 ± 0.05***
Aq.extract (8 mg)	6.55 ± 0.75***
Diclofenac (5 mg)+ Aq.extract (4 mg)	11.50 ± 0.65**
Diclofenac (5 mg)+ Aq.extract (8 mg)	8.42 ± 3.11**
sAlc.extract (4 mg)	9.00 ± 1.00***
Alc.extract (8 mg)	6.83 ± 0.79***
Diclofenac (5 mg) + Alc.extract (4 mg)	10.33 ± 0.64***
Diclofenac (5 mg) + Alc.extract (8 mg)	4.05 ± 1.33 ***

n =6 mice in each group, **P<0.01,***P< 0.001 significantly different as compared to control group.

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