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ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF HYDROALCOHOLIC EXTRACT OF FICUS MICROCARPA L. LEAVES IN EXPERIMENTAL ANIMALS

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ABSTRACT

Hydroalcoholic extract of Ficus microcarpa was screened for analgesic and anti-inflammatory activities in experimental animals. The test extract was found to be safe up to 2000 mg/ kg body weight during acute toxicity study. Hydroalcoholic extract of the test drug at doses of 250 mg and 500 mg per kg body weight showed significant analgesic activity through various models of nociception in Sprague Dawley rats and Swiss mice. The test extract was also found to possess significant anti-inflammatory activity as evidenced by carrageenan and formaldehyde hind paw oedema methods in Sprague Dawley rats.

KEYWORDS: Ficus microcarpa, leaves, hydroalcoholic extract, analgesic, anti-inflammatory.

INTRODUCTION

Ficus microcarpa L (Family: Moraceae) also known as India Laurel is an evergreen tropical tree with numerous aerial roots. It is cultivated as ornamental garden plant as well as bonsai indoor plant. Bark of Ficus macrocarpa is used in the treatment of haemorrhages, itching, hepatic disorders, itching and leprosy.[1]

The aqueous extract of leaves of Ficus macrocarpa has revealed the presence of carbohydrates, saponin, tannins, phenol and flavonoids.[2] In an experimental study, ethanolic

extract of Ficus microcarpa leaves has shown hypoglycaemic activity in alloxan induced diabetes model in Wistar rats.[3] The hexane extract of leaves was found to possess hypolipidemic and antioxidant effects in experimental hypercholesterolemia in rats.[4] The anti-diarrhoeal activities of methanolic extract of leaves of Ficus microcarpa has been experimentally demonstrated in rats.[5] In the present study hydroalcoholic extract of the leaves has been screened for analgesic and anti-inflammatory activities in experimental animals.

MATERIALS AND METHODS

Test drug

The leaves of *Ficus microcarpa* L were collected from the local areas of Cheruthuruthy, Thrissur, Kerala and were authenticated. The leaves were then dried and extracted with 50: 50 ethanol and water. The residue was removed, concentrated in rotary evaporator and were stored under refrigeration. Phytochemical studies were carried out at Quality control laboratory of the Institute.

Animals

Swiss albino mice and Sprague Dawley rats of either sex were used in the trial. The study was conducted with approval of Institute Animal Ethics Committee vide approval No. IAEC/NRIP/2014-15/02 at experimental animal facility of National Ayurveda Research Institute for Panchakarma, Cheruthuruthy, Thrissur.

Toxicity studies

During acute toxicity study the test drug was administered at dose of 2000 mg per kg body weight once orally to set of 3 female animals and the animals were observed for a period of 14 days for clinical signs of toxicity and death. The test was repeated with next set of 3 female animals at same dose level.[6]

Efficacy studies

Efficacy studies were carried out with 5 groups of animals each containing 3 male and female animals.

1. CTL- Control group (distilled water)
2. LD - Low dose group (100 mg / kg Body weight)
3. AD- Average dose group (250 mg / kg Body weight)
4. HD - High dose group (500 mg / kg Body weight)

5. STD - Standard drug group.

Evaluation of analgesic activity

(a) Tail flick method

The basal reaction time in seconds for tail flicking response was recorded in Sprague Dawley rats. Vehicle, test drugs and Standard drug (Ibuprofen – 100 mg per kg body weight) were administered to respective groups and 45 minutes post administration, tail flick response at different time intervals was recorded.[7]

(b) Hot plate method

Basal reaction time to pain stimulus were noted individually in mice by placing on a hot plate maintained at constant temperature (55^0 C) and the reaction time in seconds for showing reactions like jumping response or paw licking were noted. The reaction time post-test drug administration at different time intervals were also noted control, test groups and standard groups (Ibuprofen – 100 mg per kg body weight) as per the method of Eddy and Leimbach.[8]

(c) Formaldehyde induced paw licking method

vehicle, test drug in different doses and standard drug Indomethacin (10 mg per kg body weight) were administered orally to mice in the respective groups. Forty-five minutes post administration, 20 µl of 1% formaldehyde was injected subcutaneously under surface of the paw. The nociceptive response was recorded for 0-5 minutes (Early Phase) and 15 – 30 minutes (Late Phase) post formaldehyde injection.[9]

(d) Acetic acid writhing

The writhing test was conducted in mice, wherein 3% acetic acid was injected intraperitoneally used to induce writhing in mice 45 minutes post vehicle, test drug in different doses and standard drug (Indomethacin 10 mg per kg body weight in different doses and standard drug administration. Number of writhing response 5 minutes post acetic acid injection was counted.[10]

Evaluation of Anti-inflammatory activity

(a) Carrageenan hind paw oedema method

Basal paw volume was recorded individually in rats using plethysmometer. 45 minutes post vehicle, test drug and Standard drug (Ibuprofen – 100 mg per kg boy weight) administration were injected to the respective groups and 45 minutes later 0.1 ml of 1% Carrageenan in

normal saline was injected below plantar surface of right hind paw. The increase in paw volume was noted 3 hours post carrageenan administration by using plethysmometer and compared between the groups.[11]

(b) Formaldehyde induced hind paw oedema

Basal paw volume was recorded individually in rats using plethysmometer. 45 minutes post vehicle, test drug and Standard drug (Ibuprofen – 100 mg per kg boy weight) administration were injected to the respective groups and 45 minutes later 0.1 ml of 1% formaldehyde was injected below plantar surface of right hind paw. The increase in paw volume was noted 3 hours post carrageenan administration by using plethysmometer and compared between the groups.[12]

RESULTS

Phytochemical study

Phytochemical analysis of the hydroalcoholic extract of Ficus microcarpa revealed the presence of Proteins, carbohydrates, saponin, tannin and flavonoids.

Toxicity study

No mortality and clinical signs of toxicity were observed in Sprague Dawley rats and Swiss mice which received of hydroalcoholic extract of Ficus microcarpa L. at single oral dose of 2000 mg per kg body weight.

Tail flick method

Significant prolongation of tail flick duration was observed in the animals which received average and high dose of the test drug as compared to control group at 120 and 240 minutes of the study. (Table 1)

Table 1: Antinociceptive effect of hydroalcoholic extract of *Ficus microcarpa* on latency of tail flick response in rats.

Groups	Initial	60 Min	120 Min	240 Min
CTL	7.5±0.29	7.33±0.36	7.33±0.28	7.67±0.28
LD	7.17±0.40	7.33±0.42	7.83±0.54	8.00±0.36
AD	7.5±0.22	7.67±0.21	8.67±0.21**	8.67±0.21**
HD	6.83±0.31	7.5±0.34	8.33±0.33**	9.0±0.26***
STD	6.67±0.21	7.5±0.22	8.17±0.17***	9.33±0.21****

** P<0.01 *** P<0.001 **** P.<0001

Hot plate method

The observations recorded during the hot plate test showed the significant antinociceptive effect of average and high dose of test drug at various time intervals as compared to control. (Table 2).

Table 2: Antinociceptive effect of hydroalcoholic extract of *ficus microcarpa* on hot plate analgesia in mice.

Groups	Initial	60 Min	120 Min	240 Min
CTL	9.17±0.31	9.5±0.56	9.67±0.62	10.33±0.56
LD	9.0±0.36	9.83±0.31	10.17±0.48	10.50±0.50
AD	9.33±0.21	10.00±0.36	10.50±0.22*	10.83±0.17**
HD	9.17±0.31	9.5±0.43	10.83±0.31**	11.17±0.31**
STD	9.33±0.33	10.50±0.22*	11.00±0.26**	11.33±0.42***

*P<0.05 ** P<0.01 *** P<0.001

Formaldehyde induced paw licking method

Significant reduction in duration of paw lickings were observed in mice which received average dose and high dose of test drug as compared to control at early as well as late phase of the test (Table 3).

Table 3: Effect of Hydroalcoholic extract of *Ficus microcarpa* on paw licking test in mice.

Groups	Paw licking in seconds (Mean ±SEM)	
	Early Phase	Late Phase
CTL	42.17±1.47	134.4±2.38
LD	39.67±0.95	121.8±4.77
AD	33.50±1.72**	113±2.69**
HD	32.50±1.5***	112.4±3.14**
STD	29.33±1.71****	97.2±4.31****

** P<0.01 *** P<0.001 **** P<0.0001

(c) Acetic acid writhing test

The result shows that the extract significantly reduced the number of writhings in mice at average and high doses as compared to control. (Table 4).

Table 4: Effect of hydroalcoholic extract of *ficus microcarpa on* acetic acid induced writhing in mice.

Groups	No. of stretching episodes (Mean ±SEM)	% Protection
CTL	21.83±1.20	
LD	17.83±1.50	18.32
AD	16.50±1.52*	24.42
HD	15.33±0.67**	29.78
STD	13.50±0.50***	38.12

* P<0.05 ** P<0.01 *** P<0.001

Carrageenan induced hind paw oedema method

Significant reduction in paw volume was observed in animals which received test drug at average ($P \leq 0.01$) and high dose ($P \leq 0.001$) as compared to those in control group. (Table 5)

Table 5: Effect of hydroalcoholic extract of *ficus microcarpa* on carrageenan induced hind paw oedema in rats.

Groups	Increase in paw oedema in mL Mean ± SEM	Percentage inhibition
CTL	0.44±0.02	--
LD	0.42±0.02	4.94
AD	0.32±0.02**	27
HD	0.31±0.02***	29.28
STD	0.23±0.02****	47.91

** P<0.01 *** P<0.001 **** P<.0001

Formaldehyde induced hind paw oedema method

Significant reduction in paw volume was observed in animals which received test drug at average and high dose in dose dependent manner as compared to those in control group. (Table 6)

Table 6: Effect of Hydroalcoholic extract of *Ficus microcarpa* leaves on formaldehyde induced hind paw oedema in rats.

Groups	Increase in paw oedema in mL (Mean ± SEM)	Percentage inhibition
CTL	0.39±0.02	--
LD	0.36±0.21	8.51
AD	0.30±0.02*	22.13
HD	0.27±0.02**	31.06
STD	0.22±0.01***	44.68

* P<0.05 ** P<0.01 *** P<001

DISCUSSION

The safety of the hydroalcoholic extract of the *Ficus microcarpa* was ascertained through acute toxicity study and the single oral dose of 2000 mg per kg body weight was found to be safe in Sprague Dawley rats and Swiss mice. There was a significant increase in duration of tail flick response which indicated increase in the threshold for pain and alteration of physiological response to pain like centrally acting analgesics.[13] Similarly, animals which received the test drug at average and high doses showed significant increase in reaction time exhibited by licking and jumping response in hot plate method. This finding can be attributed to the central analgesic effect of the Ficus microcarpa extract wherein the drug might act at supra spinal level and bringing alteration of the processing and interpretation of pain impulses and inhibitory impulses to descending pathways.[14]

The hydroalcoholic extract significantly reduced the duration of paw licking consequent to injection of formaldehyde into the paw of mice in initial as well as in later stages of the study. The centrally acting analgesics reduce duration of paw licking in both phases, whereas the peripheral analgesics reduce the reaction to nociception only in the late phase.[15] It indicates the extract possess central as well as peripheral antinociceptive activities. The reduction in number of writhing in the groups which received AD and HD of the test drug further indicates the peripheral nociceptive activity.

The hydroalcoholic extract of the test drug showed significant anti-inflammatory activity in both carrageenan and formaldehyde induced hind paw oedema methods. This might be due to presence of flavonoids and tannins in the extract.[16]

CONCLUSION

The hydroalcoholic extract of Ficus *microcarpa L* showed significant antinociceptive activity in Sprague Dawley rats and Swiss mice. The centrally mediated antinociceptive effect was exhibited through significant prolongation of response to the nociceptive stimuli as demonstrated through tail flick method and hot plate method. Significant reduction in duration of licking response in formaldehyde induced paw licking test and decrease in number of stretching episodes in acetic acid induced writhing test was indicative of peripheral nociceptive activity. The extract also exhibited significant anti-inflammatory activity at the same dose levels in carrageenan and formaldehyde hind paw oedema methods.

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