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Evaluation of Methanolic Extract Induced Antinociceptive, Anti-pyretic and Anti-inflammatory Activity of *Ficus hispida* Leaves

S. M. Mushiur Rahman[1*], Md. Rubel Haque[1], Md. Ashraful Zaman[1], Sohel Rana[1], Samiron Sana[1] and Md. Tanjir Imam Hasan[1]

[1]Department of Pharmacy, Faculty of Biological Science and Technology, Jessore University of Science and Technology, Jessore-7408, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Traditionally *Ficus hispida* is used as a palliative for pain, inflammation, diabetes, fever and neurological disorders. The present study set out to evaluate the antinociceptive, anti-pyretic and anti-inflammatory activity of the methanolic extract of *Ficus hispida* leaves (MFHL).
Methods: Pithy dried leaves of *Ficus hispida* were extracted with sufficient amount of pure methanol. The antinociceptive activity of MFHL was evaluated by using formalin induced paw licking, acetic acid-induced writhing, tail immersion and hot plate methods in Swiss albino mice. Its anti-pyretic activity was evaluated by Brewer's yeast-induced pyrexia test. And anti-inflammatory activity was assessed by using xylene-induced ear edema test.
Results: The crude extract was found to have significant ($p<0.05$, vs. control) analgesic activity at the oral dose of 200 & 400 mg/kg b. wt., in the tested animals. Extract at dose of 200 mg/kg and 400

**Corresponding author: E-mail: smushiurjustphar@gmail.com;*

mg/kg significantly ($p<0.05$, vs. control) reduced paw licking and abdominal writhing of mice, and revealed significant increase in latency ($p<0.05$, vs. control) in tail immersion and hot plate test at 30 and 60 min after their administration. In Brewer's yeast- induced pyrexia test, the methanolic extract at the dose of 200 mg/kg and 400 mg/kg significantly ($p<0.05$, vs. control) reduced hyperthermia in mice in 1 hour observation and also lowering of temperature from 2 hours to 4 hours observation period respectively. In anti-inflammatory test, 200 mg/kg and 400 mg/kg of extract exhibited significant ($p<0.05$, vs. control) ear weight differences and inhibition of ear edema.
Conclusion: Results of above study indicate that *Ficus hispida* leaves can be a potential source of analgesic, antipyretic and anti-inflammatory medication.

Keywords: Ficus hispida; antinociceptive; anti-pyretic; anti-inflammatory; acetic acid-induced writhing; xylene-induced ear edema; pyrexia test.

1. INTRODUCTION

There is a remarkable correlation between pain, fever and inflammation. Increase pain and body temperature are two major signs of the body against inflammation [1]. Pain is an unpleasant emotional and sensorial modality which in many cases represents the only symptom for the diagnosis of several diseases, which disrupts the lives of millions of people throughout the world on a daily basis.

There are different reasons that may cause tissue damage including as thermal, chemical and mechanical incitements or the existence of pathologic procedure- inflammation, tumor, nerve damage, and muscle spasm. People can suffer from chronic pain during incurable diseases like rheumatoid arthritis or cancer or acute pain, which can occur through sprains or even minor injuries and accidents [2]. Pyrexia or Fever is defined as an elevation of body temperature that can be initiated by secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. A normal temperature is regulated by equilibrating heat production from muscles, hepatic metabolism, and heat loss to the environment, whereas, induction of fever is mediated by the release of pyrogenic cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF- α) [3]. Lethargy, depression, anorexia, sleepiness and inability to concentrate are the symptoms which are associated with pyrexia.

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants or damaged cells. Understanding inflammation has always been an enigma for mankind. The standard signs of inflammation are expressed by increased blood flow, elevated cellular metabolism, vasodilation, the release of soluble mediators, extravasations of fluids and cellular influx [4]. Due to the presence of the inflammatory agent, cell membranes induced the activation of phospholipase A_2 followed by the release of arachidonic acid and inflammatory mediators such as- cytokines, serotonin, histamine, prostaglandin and leukotrienes that increase vascular permeability, thus facilitating the migration of leukotrienes the site of inflammation [5].

It is also known that almost all anti-inflammatory agents possess some degree of antipyretic and analgesic activities in conjunction with toxic effects on gastric mucosa [6]. The major classes of drugs that reduce pain, cure fever and suppress inflammation are non-steroidal anti-inflammatory agents (NSAIDS) and corticosteroids but their toxic adverse effects have limited their use [7]. So, medicinal compounds that derived from plant sources such as flavonoids, saponins, alkaloids, terpenoids, glycosides and coumarins could provide an excellent fountainhead to develop new analgesic, antipyretic and anti-inflammatory agents, which could be more efficacious, safer, affordable and accessible for patients.

1.1 Plant Details

Ficus hispida is a medium but well-distributed species of tropical fig tree or shrub that is coarsely hairy and dioeciously. *Ficus hispida* is a member of the Moraceae family. It is generally known as Dumoor in Bangladesh. *Ficus hispida* is a medicinal tree, which can attain a height up to 10 meters. It is commonly a popular plant which is widely distributed throughout subcontinent from Bangladesh to India and Malaysia and is also found in Australia [8].

Astringent, antidysenteric, antipsoriasis, antianemic and antihemorrhagic properties of the

whole plant (bark, fruit, root and leaves) has already been demonstrated and reported elaborately [9,10]. The roots and leaves are comprehended for their antidiarrhoeal [11], antidiabetic [12], antibacterial [13], hepatoprotective [14], antioxidant [15] and cardioprotective [16] properties. The fruit is edible and acts as a coolant and tonic. A mixture of honey and its juice is a good antihemorrhagic [17].

Notable chemical compounds of *Ficus hispida* are ficushispimines A and B, ficushispidine, hispiloscine, β-amyrin acetate, N-triacontanyl acetate, Ficusin A, lupeol acetate and 10-keto-tetracosyl arachidate [18,19,20] which are revealed from recent publication.

Traditionally, *Ficus hispida* leaves is a regular folklore medicine in some regions of Bangladesh and used against pain, diarrhea, diabetes fever, inflammation and neurological disorders such as epilepsy and depression.

Therefore, the present study was designed to justify the antinociceptive, anti-pyretic and anti-inflammatory activities of *Ficus hispida* leaves, and evaluate the traditional usage scientifically.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The freshness leaves of *Ficus hispida* plant were collected from Jessore University of Science & Technology Campus, Jessore, Bangladesh, in September 2017. The collected leaves were identified and confirmed by National Herbarium, Bangladesh.

2.2 Preparation and Extraction of Plant Materials

The leaves of *Ficus hispida* were thoroughly washed with fresh water to remove all dirt and contaminants and dried in shade at room temperature (25±2°C) for two weeks. The materials were grinded into coarse powder and cold extraction method was used to extract the active components. The ground leaves (250 gm) were soaked in sufficient amount of methanol for 14 days at room temperature with periodical shaking and stirring. The whole mixture was primarily filtered through cotton and then through Whatman No.1 filter papers. The solvent was evaporated with a rotary evaporator under reduced pressure at 40°C temperature to yield semisolid crude extract. The extract was then preserved in a refrigerator till further use.

2.3 Experimental Animals

To perform the experiment of analgesic and antipyretic activity, one hundred and fifty Swiss albino mice (Male *Swiss albino* mice), aged 4-5 weeks, weighing about 25-30 gm were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. Before initiating the experiment, the animals were exposed to alternative 12:12 hours light and dark cycle at an ambient temperature of 26±2ºC. Proper supplies of foods and water ad *libitum* were ensured. All protocols for the animal experiment were approved by the Institutional Animal Ethical Committee of Jessore University of Science & Technology, Jessore, Bangladesh. Mice were acclimatized for 7 days in the laboratory environment prior to the study and maintained the constant environmental and adequate nutritional conditions throughout the period of the experiment.

2.4 Evaluation of Analgesic, Antipyretic & Anti-inflammatory Activity of the Methanolic Extract of *Ficus hispida* Leaves

2.4.1 Drugs and chemicals

Dimethyl sulfoxide (DMSO), Distilled water, Diclofenac sodium, Tramadol hydrochloride, 0.7% acetic acid solution, 2.7% formalin solution, Paracetamol 500 mg, Brewer's yeast, Xylene.

2.4.2 Route of administration

For analgesic tests, the extract, diclofenac sodium (100 mg/kg), tramadol hydrochloride were administered orally [per oral (p.o.) route]. 0.7% acetic acid solution was administered intraperitoneally (i.p.), and 2.7% formalin solution was administered into the dorsal surface of the left hind paw. For the antipyretic test, the extract, paracetamol (100 mg/kg) were administered orally [per oral (p.o.) route]. Brewer's yeast 15% (w/v) was injected subcutaneously (s. c.). For the anti-inflammatory test, the extract and diclofenac sodium (100 mg/kg) were administered orally [per oral (p.o.) route]. Xylene was applied on the anterior and posterior surface of the right ear lobe.

2.4.3 Preparation of the test materials and standard for the test

The crude methanol extract was triturated by the addition of small amount of suspending agent (Dimethyl sulfoxide). Distilled water was slowly added to make the concentration of the solution 400 mg/kg and 200 mg/kg. For the analgesic test, for the preparation of standard, Diclofenac sodium (100 mg) and Tramadol hydrochloride (10 mg) were dissolved into distilled water to make the concentration 100 mg/kg and 10 mg/kg respectively. For the preparation of 0.7% acetic acid solution, 0.7 mL of glacial acetic acid was mixed with distilled water to 100 mL. For the preparation of 2.7% formalin, 2.7 mL formalin was mixed with distilled water to 100 mL. For the anti-pyretic test, for the preparation of standard, (Paracetamol 500 mg) was dissolved into distilled water to make the dose 100 mg/kg. For the anti-inflammatory test, for the preparation of standard, Diclofenac sodium (100 mg) was dissolved into distilled water to make the concentration 100 mg/kg. A control sample containing DMSO in distilled water.

2.4.4 Acute oral toxicity study

Adverse effects that result either from a single exposure or from multiple exposures over a short time (normally less than 24 h) are known as acute toxicity. According to Organization of Economic Cooperation and Development (OECD) guidelines, the acute toxicity study of *Ficus hispida* leaves (Male *Swiss albino* mice) was designed to estimate the half lethal dose (LD50) of the experimental sample [21]. Fifteen mice were divided into two groups: control group and test group (MFHL), with five animals per group. The experimental sample (MFHL) was administered orally at different concentrations (100, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight). After that the animals were observed every 1 h for next 5–6 h for mortality, behavioral pattern changes such as salivation, weakness, aggressiveness, food or water refusal, diarrhea, discharge from eyes and ears, noisy breathing, changes in locomotors activity, convulsion, coma, injury, pain or any sign of toxicity in each group of animals. A final evaluation at the end of a 2-week observation period was also conducted [21].

2.4.5 Designing of the experiment

The experimental animals were randomly divided into four groups consisting of five mice (n=5) in each group. The groups were denoted from group-I to group- IV. Group I and II used as control and standard and group III and IV used as treatment groups. Each group of mice received a specific treatment. Prior to administering the drugs, each mouse was weighed properly and the doses were adjusted accordingly.

2.4.6 Formalin-induced paw licking test

The formalin-induced paw licking test was performed according to Hunskaar and Hole [22]. Twenty Swiss albino mice were selected for this test and divided into four groups containing five mice in each group, and they fasted for 16h with water ad *libitum*. Control group, standard group, and test groups were treated with distilled water (10 mL/kg), diclofenac sodium (DS, 100 mg/kg), MFHL at 200 and 400 mg/kg, respectively. All of the treatments were administered orally [per oral (p.o.) route]. After 1h of treatment, each mouse was injected with 20μL of 2.7% (v/v) formalin solution into the dorsal surface of the left hind paw. Mice were observed for 5 min after injection and the time spent in licking, biting, and shaking behaviors was measured in seconds, which was considered as acute phase (0-5 min). Again, they were monitored for 5min after 20 min of injection which was defined as late phase (20-25 min).

The percentage of inhibition of licking was calculated by the following formula.

> Inhibition (%) = [1− Licking time (standard or extracts)/ Licking time (normal control)] ×100

2.4.7 Acetic acid-induced writhing test

Analgesic activity was evaluated by the test of abdominal writhing induced by acetic acid in mice. The method of Koster et al. [22] was applied for this test. Mice were kept unfed for 16h with water ad *libitum* prior to the experiment and pretreated with extracts as mentioned before. DS (100 mg/kg) was used as a standard or positive control and distilled water as a normal control. After 45 min of respective treatment, each mouse was injected intraperitoneally with 0.7% (v/v) acetic acid at a dose of 10 mL/kg body weight. The number of writhing responses of each mouse was counted for 5 min period, which began 15 min late of acetic acid administration.

To calculate the percentage of inhibition of writhing, the following formula was used.

> Inhibition (%) = [1− No. of writhing (standard or extracts)/ No. of writhing (normal control)] ×100

2.4.8 Tail Immersion test

The tail immersion test was conducted according to Toma et al. [23]. In this experiment, a central mechanism of pain or analgesic activity can be evaluated. Thermal stimuli act as the generator of painful reaction through dipping the tail tip in hot water (55±1°C). Twenty mice were homogeneously divided into four different groups and treated as described before. Here, tramadol hydrochloride (10 mg/kg) was used as standard drug. Before and after the treatment of each mouse, the basal reaction time was counted. The counting was before 30 min and after 30, 60, 120 and 180 min of the respective treatment to determine the latency period. The animal which had more than 15 s latency periods was removed from the experiment and 15s acts as a cut-off point to avoid injury.

2.4.9 Hot plate test

Hot plate test was carried out according to the method of Turner et al. [24]. To evaluate the central mechanism of analgesic activity this method was used properly. In this assay, mice were placed on a heated (50 ± 0.05°C) metal plate. The mice showing initial reaction (lifting or licking of the paws) time of 15 s or less were selected for this test. A cut-off period of 30 s was imposed to avoid tissue damage to the paws. Mice were grouped and treated as described before and fasted for 16h with water *ad libitum*. Here, Tramadol (10 mg/kg) was used as reference drug. 30 min before the treatment of each mouse, latencies of mice were measured by placing them on the hot plate after the observation of some parameters (removal, jumping, or licking of the paws). Similarly, the response latencies were also measured after 30, 60, 120, 180 min of the respected treatment of each group. Antinociceptive activity was expressed as the increase in latency time to thermal stimulus with respect to control.

2.4.10 Brewer's yeast-induced pyrexia test

The method of Turner with slight modification was used for studying the Brewer's yeast-induced pyrexia test [25]. Forty healthy mice fasted overnight with water *ad libitum*. Before inducing pyrexia, their rectal temperatures were recorded by using an electric thermometer that was connected with a probe and inserted 2 cm into the rectum. 15% (w/v) suspension of brewer's yeast at a dose of 10 mL/kg was injected subcutaneously in the back below the nape of the neck for the induction of pyrexia. To spread the suspension under the skin of injection site was massaged thoroughly. After 18h from injection period, the increase rectal temperature was recorded, and the mice that showed an increase in temperature of at least 0.6°C were considered pyretic mice and used for brewer's yeast-induced pyrexia test. Mice were grouped and treated as described before. Here, paracetamol (100 mg/kg) used as standard drug. All treatments were given orally to the pyretic mice. Rectal temperatures of each pyretic mouse were recorded at 1, 2, 3 and 4 hours by the electric thermometer.

2.4.11 Xylene-induced ear edema test

To evaluate xylene-induced ear edema in mice Dai et al. [26] method was used. Twenty mice were divided into four groups as described before. Negative control (10 mL/kg) received one dose of distilled water, where the standard group (100 mg/kg) treated with diclofenac sodium (DS) as well as test groups received MFHL at 200 and 400 mg/kg orally. After one hour of the particular treatment, each animal received 20 μL of xylene on the anterior and posterior surfaces of the right ear lobe, where the left ear was kept untreated and considered as control. Mice were sacrificed by cutting off both ears with the utilization of 5 mm circular section after 1h of xylene application, then seized, and finally weighed. The weight of xylene-induced edema was calculated from the difference between weight of ear treated with xylene (right ear) and the weight of ear left untreated (left ear).

The percentage inhibition of ear edema was calculated by the following formula.

> Inhibition (%) = [1- Weight of edema (extract or standard drug)/ Weight of edema (normal control)] X 100

3. RESULTS AND DISCUSSION

3.1 Acute Oral Toxicity Study

No mortality was viewed up to the dose as high as 4000 mg/kg for MFHL or control group in acute oral toxicity study. Any signs of toxicity or behavioral changes were not observed up to the dose as high as 4000 mg/kg for MFHL (test group) or control group, before or after their administration in any animal, which lived up to 14 days. This apparently indicated that the test group does not show acute oral toxicity.

3.2 Evaluation of Analgesic Activity

3.2.1 Formalin-induced paw licking test

In the acute phase,
Diclofenac sodium (100 mg/kg) which was used as the standard drug, exhibited a reduction of paw licking time in the early phase (0-5 min) with a mean of (60.25±7.65) sec but failed to produce a significant result in this acute phase compared to that of control (128.85±5.67). Percent of inhibition by the standard drug (Diclofenac sodium) was 53.24% compared to that of control. Methanolic extracts of *Ficus hispida* leaves at dose 200 mg/kg and 400 mg/kg reduced paw licking time in the acute phase with a mean of (66.14±7.70) sec and (50.90±4.17) sec which were highly significant ($p < 0.05$) compared to control. Percent inhibition of both doses in acute phase were 48.67% and 60.50% respectively, compared to that of control.

In the late phase,
The standard drug (diclofenac sodium, 100 mg/kg), exhibited a reduction of paw licking time in with a mean of (2.58±0.27) sec which was very highly significant ($p < 0.0001$) compared to that of control (52.77±5.37). Percent of inhibition was 95.11 % compared to that of control. Methanolic extracts of *Ficus hispida* leaves at dose 200 mg/kg and 400 mg/kg also reduced paw licking time in the late phase (20-25 min) with a mean of (6.17±1.04) sec and (3.47±0.28) sec which were highly significant ($p < 0.05$) compared to control, and percent of inhibition were 88.31% and 93.42% which are comparable to standard drug (Diclofenac sodium).

The effect of the methanolic extract of *Ficus hispida* at 400 mg/kg was better than the 200 mg/kg dose both in the early and late phase. The results are shown in Table 1.

In this test, peripheral and central activities of nociception are revealed. Here, the response time of the animals spends in licking the injected paw were measured in both acute and late phase during this test. Two different periods of licking activity, an early response (0-5 min after the formalin injection) and a late response (20-25 min after the formalin injection). The direct effect of formalin on nociceptors and prostaglandins occurred in early phase which was not significant in the early phase (no inflammatory pain) where the late phase reflects pain from formalin-induced inflammation which can be inhibited by anti-inflammatory drugs. The late response is inhibited by peripheral analgesic only where both phases response are inhibited by the narcotic analgesic [27]. Methanolic extract of *Ficus hispida* leaves inhibited the percentage inhibition of licking at both phases.

3.2.2 Acetic acid-induced writhing test in mice

Methanolic extract of *Ficus hispida* leaves was tested for the analgesic effect using the acetic acid-induced writhing test, where the effect of the extract was measured by the inhibition of the number of writhing induced by acetic acid in mice. 200 mg/kg and 400 mg/kg concentrations of the methanolic extract were tested. The effects are displayed in Table 2. Diclofenac sodium (100 mg/kg) was used as a standard.

The standard drug, diclofenac sodium (100 mg/kg) was very effective in reducing the number of writhing in mice induced by the administration of acetic acid. The mean number of writhing was 13.20±1.74, which was very highly significant ($p = 0.000$) compared to that of the control (26.20±2.35).

Methanolic extract at both 200 mg/kg and 400 mg/kg doses highly inhibited the number of writhing, which proves that the extract could have analgesic activity. The extract was more effective at high dose (400 mg/kg) with a mean value of 14.60±2.18 than the low dose (200 mg/kg) with a mean value of 19.40±1.33. But both of their results were very significant ($P= 0.001$, $P= 0.031$ in respectively). Percent protection offered by 200 and 400 mg/kg were 25.95 and 44.27% respectively.

In the acetic acid-induced writhing experiment, the pain sensation is arising through the activation of the localized inflammatory response by acetic acid. Here endogenous substances such as serotonin, bradykinin, histamine and prostaglandin are involved in pain generation [28, 29]. Therefore, plant extract might be inhibiting the synthesis and /or release of these endogenous substances and thus reduce pain.

3.2.3 Tail immersion test

Tail immersion model is one of the most significant acute pain model. This model is used for the assessment of the mechanism of central analgesics which are opioids in nature. Supraspinal (k3, d1, and s1) and spinal (k1, d2 and s2) receptors play an important role in the

analgesic activities of opioids. Thermal nociceptive tail immersion test gets additional sensitivity from opioid *μ* receptor agonists [30].

After 30 min of administration, tramadol group and 400 mg/kg MFHL had a significant increase in latency ($p < 0.05$, versus control). The maximum effects of extracts were obtained at 30 min ($p < 0.05$, versus control). It was also observed that all of the extracts (both 200 mg/kg and 400 mg/kg) and tramadol showed significant response latency at 30, 60, 120, 180 min, respectively ($p<0.05$, versus control). Table 3 shows the effects of latency time in the tail immersion test.

3.2.4 Hot plate test

The methanolic extract of *Ficus hispida* leaves produced a highly significant ($p < 0.05$) increase in the latency response of mice to hot plate thermal stimulation. The maximum effects of the extracts were obtained at 30 minutes ($p<0.05$, versus control). After the administration, it was observed that all of the extracts (both 200 mg/kg and 400 mg/kg) and tramadol showed significant response latency at 30, 60, 120, 180 min, respectively ($p<0.05$, versus control). The analgesic effect of *Ficus hispida* leaves in hot plate test are shown in Table 4.

Table 1. Effects of methanolic extracts of *Ficus hispida* leaves on formalin-induced paw licking test

Treatment Group	Dose	Acute phase		Late phase	
		Licking time (s)	% inhibition	Licking time (s)	% inhibition
Control (Vehicles)	10 mL/kg	128.85±5.67	-	52.77±5.37	-
Standard (Diclofenac sodium)	100 mg/kg	60.25±7.65	53.24	2.58±0.27	95.11
Methanolic extract	200 mg/kg	66.14±7.70	48.67	6.17±1.04	88.31
Methanolic extract	400 mg/kg	50.90±4.17	60.50	3.47±0.28	93.42

Numbers of licking time inhibition are presented as (mean ± standard error of the mean). P<0.05, vs. control; (Dennett's t-test)

Table 2. Effects of methanolic extracts of *Ficus hispida* leaves on Acetic acid-induced writhing test in mice

Treatment groups	Dose mg/kg	No of writhing	% of inhibition	*p*-value
Control (Vehicles)	10 mL/kg	26.20±2.35	-	
Standard (Diclofenac sodium)	100 mg/kg	13.20±1.74**	49.62	.000
Methanolic extract	200 mg/kg	19.40±1.33*	25.95	.031
Methanolic extract	400 mg/kg	14.60±2.18**	44.27	.001

*Number reductions of writhing are presented as (mean ± standard error of the mean). *p <0.05, vs. control; **p <0.001, vs. control (Dennett's t-test)*

Table 3. Effects of methanolic extracts of *Ficus hispida* leaves on Tail immersion test in mice

Group	Dose	Latency time (sec)				
		0 min	+30 min	+60 min	+120 min	+180 min
Control	10 mL/kg	1.93±0.14	2.05±0.12	2.04±0.06	1.89±0.07	1.59±0.13
Tramadol	100 mg/kg	2.06±0.19	5.01±0.42***	5.57±0.40***	4.31±0.27***	2.51±0.16*
Methanolic extract	200 mg/kg	1.89±0.22	2.97±0.06*	3.38±0.33**	2.48±0.19*	1.73±0.07
Methanolic extract	400 mg/kg	1.76±0.17	4.10±0.20***	4.42±0.26***	2.78±0.19*	1.94±0.31

*Response latency values are presented as mean ± standard error of mean. 0min mean 30 min before drug administration; +30 min, +60 min, +120 min, and +180 min indicate 30, 60, 120, and 180 min after drug administration, respectively. *P< 0.05, vs. control; **p<0.01, vs. control; ***p<0.001 vs. control (Dunnett's t test)*

Table 4. Effects of methanolic extracts of *Ficus hispida* leaves on Hot plate test in mice

Group	Dose	Latency time (sec)				
		0 min	+30 min	+60 min	+120 min	+180 min
Control	10 mL/kg	1.91± 0.15	1.92± 0.05	1.92± 0.22	1.98±0 .14	1.56±0 .12
Tramadol	100 mg/kg	1.92± 0.17	4.10± 0.14*	4.32±0 .17*	3.76± 0.21	2.44±0 .16
Methanolic extract	200 mg/kg	1.96±0 .10	2.62± 0.20	2.70±0 .15	2.10±0 .16	1.80± 0.10
Methanolic extract	400 mg/kg	2.04± 0.06	3.50±0 .24*	3.20±0 .26*	2.75±0 .23	1.95±0 .14

Response latency values are presented as mean ± standard error of mean. 0 min mean 30 min before drug administration; +30 min, +60 min, +120 min, and +180 min indicate 30, 60, 120, and 180 min after drug administration, respectively. $*P< 0.05$, versus control (Dunnett's t test)

Table 5. Effects of methanolic extracts of *Ficus hispida* leaves on brewer's yeast induced pyrexia test

Treatment Group	Dose	Temperature					
		Initial	0h	1h	2h	3h	4h
Control (Vehicles)	10 mL/kg	34.66±.23	35.72±.18	35.60±.18	35.50±.18	35.36±.17	35.00±.18
Standard (paracetamol)	100 mg/kg	34.78±.26	35.74±.27	34.36±.24	34.66±.21	34.54±.25	34.32±.14
Methanolic extract (200mg/kg)	200 mg/kg	34.78±.25	35.86±.30	33.68±.67	34.84±.07	34.34±.32	34.28±.19
Methanolic extract (400mg/kg)	400 mg/kg	34.56±.12	35.70±.27	34.20±.08	34.34±.30	34.30±.23	34.20±.20

Thermal values are presented as mean ± standard error of mean. P<0.05, vs. control (Dunnett's t-test).

Table 6. Effects of methanolic extracts of *Ficus hispida* leaves on xylene-induced ear edema test

Treatment Group	Dose mg/kg	Ear weight difference (mg)	Inhibition (%)	Significance
Control (vehicles)	10 mL/kg	12.00±0.71	-	-
Diclofenac sodium	100 mg/kg	5.80±0.67	51.67	.000
Methanolic extract	200 mg/kg	9.00±0.55	25.00	.003
Methanolic extract	400 mg/kg	6.80±0.37	43.33	.000

Ear weight differences are denoted as mean ± standard error of mean. $P< 0.05$ versus control (Dunnett's t test).

The hot plate test in mice is commonly used for assays of narcotic analgesics [31]. The results suggest that the plant extract has a central analgesic effect as evidenced by an increase in reaction time of mice in the hot plate test.

3.3 Evaluation of Antipyretic Activity Using Brewer's Yeast Induced Pyrexia Test

The anti-pyretic activity of the methanolic extract of *Ficus hispida* leaves has been shown in Table 5. The subcutaneous injection of yeast markedly increased the rectal temperature and the mean increment recorded was 1.24– 2°F after 18 hours of administration. The methanolic extract showed significant activity at 200 mg/kg and 400 mg/kg dose levels. The results were comparable to that of Paracetamol, a prototype of an anti-pyretic drug.

Pyrexia is thought to be produced by several endogenous substances including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), macrophage protein-1 (MIP-1) and prostaglandins [32]. Brewer's yeast is an exogenous pyrogen which initiates the synthesis and release of both TNF-α

and prostaglandins by activation of the arachidonic pathway which can easily cross the blood-brain barrier and then increases body temperature [33]. Non-steroidal anti-inflammatory drugs (NSAIDs) reduce fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulatory sites. These agents suppress peripheral production of pyrogenic cytokines such as TNF-α and interleukin-1β while lowering the thermoregulatory set point by blocking central cyclooxygenase production of prostaglandin E2 [34].

The methanolic extract of *Ficus hispida* showed a more pronounced effect in lowering the hyperthermia by inhibiting the enzyme cyclooxygenase and reducing the levels of PGE2 within the hypothalamus [35]. Recently, other mechanisms of action for antipyretic drugs have been suggested, including their ability to reduce proinflammatory mediators, enhance anti-inflammatory signals at sites of injury, or boost antipyretic messages within the brain.

3.4 Evaluation of Anti-inflammatory Activity Using Xylene Induced Ear Edema Test in Mice

The result of the anti-inflammatory activity of MFHL on topical xylene-induced ear edema in mice is shown in Table 6. The cutaneous inflammation of mouse was rapidly obtained after xylene induced. All of the groups showed significant (P<0.05 versus control) inhibition of ear edema and differences of ear weight. Among the extracts, 43.33% is the highest value of inhibition that was observed by MFHL 400 mg/kg.

Inflammation has four cellular processes, which are changes in blood flow by changing in smooth muscle cell function that is accountable for vasodilatation, alteration of the vascular permeability, migration phagocytic leukocytes to the site of inflammation, and phagocytosis [36]. Xylene-induced ear edema test is done as an acute inflammatory test. In addition, xylene can release inflammatory mediators such as bradykinin, histamine, and serotonin. These mediators are responsible for edema as they enhance vascular permeability and improve vasodilation [37]. Fluid accumulation occurs at the treatment site, which is shown by the xylene-induced ear edema test and inhibition of this fluid accumulation is considered as anti-inflammatory effect [38]. Diclofenac sodium is a cyclooxygenase inhibitor. It inhibits prostaglandin synthesis and somewhat cycloxygenase-2 selective. The methanol extract has an activity which is comparable to Diclofenac sodium can be said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity [39]. Here, the leaves of *Ficus hispida* contains β-amyrin acetate, phenolic and flavonoids compounds which produced significant inhibition of ear edema, that may be due to the blockage of phospholipase A2, reduction of vascular permeability, and vasodilation and reduce inflammation [40,41]. But extensive study is required to assure the exact mechanism, through which the extracts suppressed edema.

4. CONCLUSION

From the earlier [39] and existing study, it could be suggested that Methanolic extract of *Ficus hispida* leaves might possess remarkable analgesic, antipyretic and anti-inflammatory properties. Data obtained in this study showed that all activities were dose-dependent and statistically significant. The presence of flavonoids, β-amyrin acetate, lupeol acetate, and phenolic compounds might be responsible for these activities and which are probably mediated via inhibition of various autocoids formation and release. We hope that, further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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