



Pharmacological evidence for the use of *Cissus assamica* as a medicinal plant in the management of pain and pyrexia

Trishala Dutta^{a,b}, Arkajyoti Paul^{c,d}, Mohuya Majumder^{c,e}, Ramiz Ahmed Sultan^b, Talha Bin Emran^{c,f,*}

^a Department of Pharmacy, City University, 13/A Panthapath, Dhaka, 1215, Bangladesh

^b Department of Pharmacy, Faculty of Biological Science, University of Chittagong, Chittagong, 4331, Bangladesh

^c Drug Discovery, GUSTO A Research Group, Chittagong, 4000, Bangladesh

^d Department of Microbiology, Jagannath University, Dhaka, 1100, Bangladesh

^e Department of Pharmacy, East West University, Dhaka, 1212, Bangladesh

^f Department of Pharmacy, BGC Trust University Bangladesh, Chandanaish, Chittagong, 4381, Bangladesh

ARTICLE INFO

Keywords:

Cissus assamica
Vitaceae
Phytochemicals
Antinociceptive
Antipyretic

ABSTRACT

The existing therapeutic agents for the management of pain and pyrexia are not very efficient and accompanied by numerous side effects. Thus, new effective agents are the most needed. The present study investigates bioactivities and phytochemical screening of different parts of *Cissus assamica* (Vitaceae), a Bangladeshi tribal medicinal plant. Three plant parts stems, leaves and roots were collected, washed, dried, powdered and then prepared for cold extraction. The methanolic stems and leaves extracts were fractionated with four and two solvents respectively. Different plant extracts were then investigated for *in vivo* antinociceptive activity and only methanolic leaves extract was investigated for *in vivo* antipyretic activity. In Swiss-albino mice, 200 and 400 mg/kg body weight doses were used for all extracts. In the peripheral antinociceptive activity, the methanolic stem extract and its dichloromethane, chloroform, pet ether fractions and methanolic roots extract at their both doses showed significant antinociceptive responses when compared to standard diclofenac sodium (60.49% inhibition). In the central antinociceptive activity, the response was found significant for methanolic stem and methanolic roots extract in their both doses compared to standard morphine. In antipyretic activity, methanolic leaves extract significantly reduced pyrexia level at 400 and 200 mg/kg body weight doses after two, three and 4 h of administration when compared to standard. So our findings indicate that this plant possesses noteworthy pharmacological activities which may be a basis for further researches to establish a possible mode of action of its different parts.

1. Introduction

Pain is a physical discomfort within the human body that is usually caused by a harmful stimulus [1]. Pain killers that are used traditionally to relieve pain that mainly work due to the presence of different plant metabolites in them which are responsible for the antinociceptive response. Fever, also called pyrexia, is caused due to different diseased conditions, infections or inflammation [2]. The infected, as well as damaged tissue, usually initiates the increased formation of chemical mediators that causes inflammation. Mediators such as cytokines enhance prostaglandin E2 (PGE2) synthesis near the hypothalamic area in the brain and trigger hypothalamus to elevate the temperature of the body [3]. Increased body temperature is then governed by the nervous

system feedback mechanism that causes dilation of the blood vessels and sweating to reduce the temperature. From ancient times, many medicinal plants are used traditionally for the treatment of pyrexia as phytopharmaceuticals have fewer or no side effects at all.

Pharmacological investigations of different plant extracts revealed medicinal properties of many botanicals, but yet a lot of medicinal plants remain out of investigations. Hence isolation and characterization of healing compounds from these medicinal plants were needed. According to World Health Organization (WHO), medicinal plants which are using mainly for the preparation of different herbal medicines are curing diseases of an estimated 1.5 billion (currently about 3.5 billion, i.e. 88%) of the world population [4]. About 25% of today's prescribed medicines in the world is originated from medicinal plants

* Corresponding author. Department of Pharmacy, BGC Trust University Bangladesh, Chandanaish, Chittagong, 4381, Bangladesh.

E-mail addresses: trishala.pharmacy@gmail.com (T. Dutta), arka.bgctub@gmail.com (A. Paul), mohuyamajumderbgctub@gmail.com (M. Majumder), ramiz@cu.ac.bd (R.A. Sultan), talhabmb@gmail.com, talhabmb@bgctub.ac.bd (T.B. Emran).

<https://doi.org/10.1016/j.bbrep.2019.100715>

Received 8 September 2019; Received in revised form 9 November 2019; Accepted 1 December 2019

2405-5808/ © 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

[5] and about 80% of the population relies on traditional plant-based medicines in developing countries [6]. In Bangladesh, huge medicinal plants are available mostly in the hill tract districts and used by the local tribes e.g., Chakmas, Marmas etc. [7].

Cissum assamica (Lawson) Craib known as Amasha lata locally and Sarba amila or Murmuijja amila tribally is a species belonging to the family Vitaceae. It is a large woody climber and its stems are angular and bear reddish spots; leaves are roundly cordate or orbicular, cuspidate; flowers are minute in slender cymes and in axillary leaf opposed umbellate cymes; fruits are turbinate, size of a pea, black [7]. It is available at Chittagong Hill Tracts areas as well as Cox's Bazar [7].

Through preliminary chemical investigations, about eight chemical compounds of this plant such as ursolic acid, lupeol, n-hexacosinic acid, isolariciresinol-9-O-beta-D glucopyranoside, dauco sterin, 3,3'-dimethyl ellagic acid, beta-sitosterol, bergenin were isolated [8], but further and wide chemical investigations of this plant parts are required to become well-established. Its root extract is used in the treatment of hysteria and stem is used in dysentery treatment in Khagrachari. In the treatment of jaundice, the whole plant is locally used in Rangamati [7].

Depending upon a few literatures obtained and the local data survey, these plant parts were selected for phytochemical screening as well as evaluation of antinociceptive and antipyretic activities.

2. Materials and methods

2.1. Collection and identification of plant materials

After a long survey within the tribal communities of Chittagong Hill tracts areas (Rangamati Hill District), Bangladesh, this plant *C. assamica* was selected due to its huge local medicinal uses. Sufficient amount of its stems, leaves and roots was then collected in August 2017. Later, plant parts were identified by botany expert Dr. Shaikh Bokhtear Uddin, Taxonomist and Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh by preparing herbarium specimens. A voucher specimen (accession no. 1110) has been preserved in the institutional herbarium of the aforesaid department.

2.2. Drying and grinding

Plant parts were washed properly with tap water and cut into small pieces. The pieces were then air-dried first and then oven-dried at 65 °C for 24 h to cause proper grinding. All the fractions were prepared with the same process. Then ground powdered plant materials were preserved in a dry and non-moist environment and prepared for crude extraction.

2.3. Preparation of crude methanolic extracts

About 1.5 kg of powdered stems, 500 gm of powdered leaves and 200 gm of powdered roots were taken in three different round-bottomed flasks and soaked with 8 L, 2.5 L and 1.5 L of methanol respectively. Different weights were used as availability of plant parts was limited, and not all parts were collected in the same amount due to environmental circumstances. Solvent volume was thus changed depending on the amount of dried plant parts used. Each container was then covered with aluminum foil and kept for 12 days with regular shaking and stirring. Filtration of the contents of each container was then done with white clean and pure cotton material. Hence, drying of the filtrate obtained from each container was occurred using vacuum rotary evaporator. Thus, about 25 gm of crude stem extract, 15 gm of crude leaves extract and 5 gm of crude roots extract were obtained.

2.4. Drugs and chemicals

The chemicals and solvents used in this total study were of analytical grade and all were obtained from Merck, Germany. Acetic acid,

Tween-80, Methanol, normal saline (Incepta Pharmaceutical Ltd., Bangladesh) were used. Morphine and Brewer's yeast powder were purchased from Gonoshasthaya Pharmaceuticals Ltd., Dhaka-1210, Bangladesh. Morphine, Diclofenac sodium (ACI Pharmaceuticals Ltd., Bangladesh) and Paracetamol (Square Pharmaceutical Ltd., Bangladesh) were used as standard drugs.

2.5. Experimental animals

Swiss-albino mice aged 5–6 weeks, weighing about 20–25 gm, either male or female, each obtained from the Animal House of Jahangirnagar University, Savar, Dhaka-1342 were used for this experiment. Mice were kept in clean and dry animal cages with proper light-dark cycle supply and temperature of (25 ± 2) °C and relative humidity of 45–55% were supplied for them in the Animal House of the Biological Faculty, University of Chittagong. Mice were fed with laboratory standard diet appropriate for their body conditions and allowed with drinking water *ad libitum*. These animals are very reactive to different environmental changes, so to get appropriate results they were kept minimum for 8–10 days in the experimental environment before carrying out the tests. Experiments involving animals were approved by the Institutional Animal Ethics Committee (ref no. ERC/DP/CU/2016/0021) of the Department of Pharmacy, Faculty of Biological Science of the University of Chittagong, Bangladesh.

2.6. Acute toxicity studies

An acute oral toxicity study was performed according to the OECD guidelines for the testing of chemicals, Test No. 423 (OECD, 2001; Acute oral toxicity-acute toxic class method). A total of five animals used in the toxicity study received a single oral dose of either 500 mg/kg body weight (b.w.), 1000 mg/kg b.w., 1500 mg/kg b.w. or 2000 mg/kg b.w. of *C. assamica* extract. Following dosing, food was withheld for 3–4 h. The individual animals were closely observed during the first 30 min after dosing, periodically for the first 24 h, after that for 3 days to record any delayed toxicity. Other changes, including in skin and fur, eyes and mucous membranes, respiratory and circulatory rate or autonomic and central nervous system function were observed. The median lethal dose was used (LD50 > 2.0 g/kg) to calculate the effective therapeutic dose.

2.7. Phytochemical screening

The presence of different plant metabolites like flavonoids, saponins, glycosides, reducing sugars, alkaloids, phenols, tannins, proteins, terpenoids etc. was screened in crude methanolic extract of stems, leaves and roots as well as their different partitioning fractions by using standard procedures for phytochemical screening [9,10].

2.8. Peripheral antinociceptive activity

The peripheral antinociceptive activity of different plant extracts was evaluated by using acetic acid induced writhing method in Swiss albino mice [11,12]. Ninety mice were randomly selected and divided into eighteen groups consisting of 5 mice in each group. Group-I was the control group and treated with 1% Tween-80 in normal saline and Group-II received standard drug treatment Diclofenac sodium (50 mg/kg body weight). Group-III, Group-III, Group-IV, Group-IVB, Group-VA, Group-VB, Group-VIA, Group-VIB, Group-VIIA, Group-VIIB, Group-VIIIA, Group-VIIIB, Group-IXA, Group-IXB, Group-XA, Group-XB received specific particular treatments i.e., methanolic stem extract 400 and 200 mg/kg; its dichloromethane soluble fractions 400 and 200 mg/kg, chloroform soluble fractions 400 and 200 mg/kg, pet ether soluble fractions 400 and 200 mg/kg, n-hexane soluble fractions 400 and 200 mg/kg; methanolic leaves extract 400 and 200 mg/kg; its dichloromethane soluble fractions 400 and 200 mg/kg, chloroform

soluble fractions 400 and 200 mg/kg respectively. Thirty minutes after the administration of all treatments, acetic acid (0.7%) was injected intraperitoneally to each animal of all groups to create pain sensation. Here interval between test samples and acetic acid administration was given so that the optimum level of absorption could occur. This might lead to contract their body as a result of pain which is called "writhing". Five minutes after acetic acid administration, numbers of writhing were recorded for 15 min for each mouse. Extracts that exhibit antinociceptive activity will decrease the number of writhing in animals in comparison to the control group. The percentage of writhing inhibition was then calculated by using the following equation [13]:

$$\text{Writhing inhibition (\%)} = [(Wc - Wt) / Wc] \times 100$$

Where Wt is the average number of writhing in test groups and Wc is the average number of writhing in the control group.

2.9. Central antinociceptive activity

The central antinociceptive activity of different plant extracts was evaluated by using the tail-immersion test in Swiss albino mice model [14–16]. Eighty mice were selected and divided into sixteen groups consisting of 5 mice in each group. Group-I served as a control group and Group-II received standard treatment Morphine (50 mg/kg body weight). Group-III, Group-IIIb, Group-IVa, Group-IVb, Group-Va, Group-Vb, Group-VIa, Group-VIb, Group-VIIa, Group-VIIb, Group-VIIIa, Group-VIIIb, Group-IXa, Group-IXb received specific particular treatments orally i.e., methanolic stem extract 400 and 200 mg/kg; its dichloromethane soluble fractions 400 and 200 mg/kg, chloroform soluble fractions 400 and 200 mg/kg; methanolic leaves extract 400 and 200 mg/kg; its dichloromethane soluble fractions 400 and 200 mg/kg, chloroform soluble fractions 400 and 200 mg/kg; methanolic roots extract 400 and 200 mg/kg respectively.

At zero time, about 3–5 cm of the tail of each mouse of each group was dipped into a water bath full of warm water. The temperature of the water was maintained at about (55 ± 0.5) °C. The time taken for the mouse to flick its tail from the warm water was termed as pain reaction time for that mouse. Baseline latency (reaction time) was obtained taking three measurements; the mean of these three measurements is called pre-drug latency time. A cut off period of 15–20 s is observed as time above this period would cause tissue damage to the tail. After baseline, mice of each group were treated with their respective treatments and then again the latent period of the tail-flick response of each mouse was determined 30, 60, 90 and 120 min after the administration of samples respectively [17]. The percentage of the maximal possible effect (% MPE) was calculated using the following equation [18]:

$$\frac{(\text{Post drug latency} - \text{Pre drug latency})}{(\text{Cut off time} - \text{Pre drug latency})} \times 100$$

The percentage of time elongation was also calculated from the following equation [19]:

$$\frac{\text{Latency of test sample} - \text{Latency of control}}{\text{Latency of test}} \times 100$$

2.10. Antipyretic activity using Brewer's yeast induced pyrexia mice model

The *in vivo* antipyretic activity was carried out by Brewer's yeast induced pyrexia in mice model and it was described by Al Ghamdi [20]. Thirty mice were randomly taken and identified numerically. Then initial normal body temperature of all of the mice was recorded using a digital thermometer [21]. The temperature (36.8 ± 0.7) °C is considered as normal for mice. A temperature above 37.5 °C will be considered as fever. After measurement of normal temperatures, pyrexia in all mice was induced by injecting 20% of aqueous Brewer's yeast

suspension (10 ml/kg) subcutaneously into the dorsum regions of the animals. Animals were then fasted overnight giving water only. After 24 h of yeast administration, the rectal temperature of each mouse was observed and recorded. Mice not showing a minimum increase of 0.5 °C in temperature were discarded. Twenty fasted mice with pyrexia among them were then selected and divided into four groups each of five mice. Group-I served as a control group treated with 1% Tween-80 in normal saline and Group-II received standard drug treatment Paracetamol (150 mg/kg body weight). Group-III and Group-IV received methanolic leaves extract 400 and 200 mg/kg doses respectively. Then the rectal temperature of each mouse of each group was recorded periodically after 1, 2, 3, 4 and 5 h of the administration. The percent reduction in pyrexia was calculated by the formula below:

$$\text{Percent reduction} = (B - Cn/B - A) \times 100$$

Where, B represents temperature after pyrexia induction; Cn temperature after 1, 2, 3, 4 and 5 h and A, normal body temperature.

2.11. Statistical analysis

Details concerning the study outline, sample size, and statistical analysis are shown in the main text, figures, and figure legends. Data were expressed as mean ± SEM (Standard error of the mean). The statistical analysis was performed by using either the statistical software package for social science (SPSS, version 20.0, IBM Corporation, NY), Prism version 7.0a (GraphPad Software Inc., La Jolla, CA, USA) or Microsoft® Excel (Redmond, WA, USA). Data analysis among the groups was compared using one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test. P-value < 0.001 was considered statistically significant in all cases.

3. Results

3.1. Acute toxicity studies

All the extracts and fractions of the plant *C. assamica* did not cause any mortality up to 2000 mg/kg dose level. Therefore, 200 and 400 mg/kg doses were selected for further experiments.

3.2. Phytochemical screening

The phytochemical screening ensured that different extracts of *C. assamica* plant parts contain different primary and secondary metabolites (Table 1). Almost all extracts were noted with the presence of alkaloid, steroids, tannins and flavonoids extensively.

3.3. Peripheral antinociceptive activity

The results of peripheral antinociceptive activity by acetic acid induced writhing test were shown in Table 2. The methanolic stem extract dose-dependently induced a significant (P < 0.001) decrease in a number of writhing with 78.54% and 58.78% of inhibition at both 400 and 200 mg/kg body weight doses respectively when compared to that of standard diclofenac sodium (60.49% inhibition, P < 0.001). The dichloromethane soluble fraction of stem extract at both 400 and 200 mg/kg body weight doses exhibited significant antinociceptive activity (77.56% and 58.05% inhibition of writhing respectively). The chloroform and pet ether soluble fractions of stem extract at both 400 and 200 mg/kg body weight doses also exhibited significant antinociceptive activities with (57.56% and 40.98%) and (49.76% and 32.68%) of inhibition respectively. The n-hexane soluble fraction of stem extract at both 400 and 200 mg/kg body weight doses exhibited lower antinociceptive activity (23.41% and 12.20% respectively). The methanolic leaves extract dose-dependently induced a significant (P < 0.001) decrease in the number of writhing with 43.41% and

Table 1
Phytochemical screening of different extracts of different plant parts of *C. assamica*.

Test name	Test methods	Name of plant extracts						
		MS	DFS	CFS	ML	DFL	CFL	MR
Alkaloid	Wagner's test	+	+	+	+	+	+	-
Tannin	General test	+	+	+	-	-	+	+
Steroid	Salkowski reaction test	+	+	-	+	-	-	-
Triterpenes	Salkowski reaction test	-	-	+	-	-	+	-
Flavonoids	Shinoda's test	+	+	-	+	-	-	+
Saponin	Shake or foam test	-	-	-	+	-	+	+
Resin	General test	-	+	+	-	-	+	-
Glycoside	Sodium hydroxide test	+	+	+	-	-	+	-
Cardiac glycoside	Keller-Killiani test	-	-	-	-	-	-	-
Anthraquinone glycoside	Hydroxy anthraquinone test	+	-	-	-	-	-	+
Phenol	Ferric chloride test	-	-	-	-	-	+	+
Reducing sugar	Fehling's test	+	-	-	-	-	-	-
Carbohydrate	Molisch's test	+	+	+	-	-	-	-
Protein	Biuret test	-	-	-	-	-	-	+
Fats & fixed oils	General test	-	-	-	+	+	+	-

Here (+) = presence of constituents; (-) = absence of constituents; MS = Methanolic stem extract; DFS = Dichloromethane fraction of stem extract; CFS = Chloroform fraction of stem extract; ML = Methanolic leaves extract; DFL = Dichloromethane fraction of leaves extract; CFL = Chloroform fraction of leaves extract; MR = Methanolic roots extract.

Table 2
Peripheral antinociceptive activity of different extracts of *C. assamica* by acetic acid induced writhing test.

Animal Group	Dose (mg/kg)	Number of writhing (Mean \pm SEM)	% inhibition of writhing
Control	-	41.00 \pm 2.28	-
Standard	50	16.20 \pm 0.92***	60.49
MS 400	400	8.80 \pm 0.58***	78.54
MS 200	200	16.9 \pm 2.20***	58.78
DFS 400	400	9.20 \pm 0.86***	77.56
DFS 200	200	17.20 \pm 1.24***	58.05
CFS 400	400	17.40 \pm 1.60***	57.56
CFS 200	200	24.20 \pm 1.24***	40.98
PFS 400	400	20.60 \pm 1.63***	49.76
PFS 200	200	27.60 \pm 1.94***	32.68
NFS 400	400	31.40 \pm 3.19**	23.41
NFS 200	200	36.00 \pm 2.30	12.20
ML 400	400	23.20 \pm 2.67***	43.41
ML 200	200	26.80 \pm 2.44***	34.63
DFL 400	400	28.80 \pm 1.66***	29.76
DFL 200	200	32.60 \pm 1.75*	20.49
CFL 400	400	18.00 \pm 1.41***	56.10
CFL 200	200	30.40 \pm 2.44**	25.85

Each value represents mean \pm SEM (n = 5). One-way ANOVA followed by Dunnett's *t* test. ***P < 0.001, **P < 0.01, *P < 0.05 compared with the control. MS = Methanolic stem extract; DFS = Dichloromethane soluble fraction of stem extract; CFS = Chloroform soluble fraction of stem extract; PFS = Pet ether soluble fraction of stem extract; NFS = N-hexane soluble fraction of stem extract; ML = Methanolic leaves extract; DFL = Dichloromethane soluble fraction of leaves extract; CFL = Chloroform soluble fraction of leaves extract.

34.63% of inhibition at both 400 and 200 mg/kg body weight doses respectively. The dichloromethane and chloroform soluble fractions of leaves extract at both 400 and 200 mg/kg body weight doses exhibited dose-dependent moderate antinociceptive activities with (29.76% and 20.49%) and (56.10% and 25.85%) of inhibition respectively.

3.4. Central antinociceptive activity

The results of central antinociceptive activity by the tail immersion test were shown in Table 3 and Table 4. The percent elongation of tail flicking time was calculated for the control in respective time. Higher elongation percentage indicates greater central antinociceptive activity. The results showed that the methanolic stem extract produced significant (P < 0.001) 51.90%, 55.52%, 54.97% and 46.53% and 41.60%, 42.48%, 39.86% and 35.09% elongation of tail flicking time at 30, 60, 90 and 120 min respectively after administration of 400 and 200 mg/kg body weight doses. The percent elongation of latency time was increased with time in both doses but attained the peak level at 60 min (55.52%) which is nearer to that of standard morphine (64.99%) and at 60 min (42.48%) which is almost nearer to that of morphine for 400 and 200 mg/kg body weight doses respectively. So the methanolic stem extract increased the onset time of pain sensation significantly (P < 0.001) when compared to morphine solution.

The dichloromethane soluble fraction of stem extract produced 43.95% (P < 0.001), 43.75% (P < 0.001), 39.65% (P < 0.001) and 38.33% (P < 0.001) & 23.63% (P < 0.01), 26.09% (P < 0.01), 24.23% (P < 0.001) and 24.18% (P < 0.001) elongation of tail flicking time at 30, 60, 90 and 120 min respectively after administration of 400 and 200 mg/kg doses respectively. The % elongation of tail flicking time was increased with time for 400 mg/kg body weight dose but attained the peak level at 30 min (43.95%) which is nearer to that of standard morphine (58.26%), whereas it was slowly increased with time in case of 200 mg/kg body weight dose and attained the peak level at 60 min (26.09%) which is comparable to that of the standard (64.99%).

The % elongation of tail flicking time of chloroform soluble fraction of stem extract was increased with time in case of 400 mg/kg body weight dose and attained the peak level at 60 min (46.69%) which is nearer to that of standard morphine (64.99%), whereas it was slowly increased with time and then again decreased in case of 200 mg/kg body weight dose and attained the peak level at 60 min (27.14%) which is low central analgesic activity.

The methanolic leaves extract produced 49.64% (P < 0.001), 48.83% (P < 0.001), 47.88% (P < 0.001) and 48.47% (P < 0.001) and 22.78% (P < 0.05), 32.60% (P < 0.001), 23.21% (P < 0.001) and 14.75% elongation of tail flicking time at 30, 60, 90 and 120 min respectively after administration of 400 and 200 mg/kg body weight doses respectively. The % elongation of tail flicking time was variable in case of 400 mg/kg body weight dose and attained the peak level at 30 min (49.64%) which is quite nearer to that of standard morphine (58.26%), whereas it was slowly increased and then again decreased with time in case of 200 mg/kg body weight dose and attained the peak level at 60 min (32.60%) which is comparable to that of standard (64.99%).

The % elongation of tail flicking time of dichloromethane soluble fraction of leaves extract was increased and then again decreased with time in case of 400 mg/kg body weight dose and attained the peak level at 60 min (38.55%) which is comparable to that of standard (64.99%), whereas it was slowly increased and then decreased with time in case of 200 mg/kg body weight dose and attained the peak level at 60 min (26.44%) which is low central analgesic activity.

The % elongation of tail flicking time of chloroform soluble fraction of leaves extract was increased and then decreased with time in case of 400 mg/kg body weight dose and attained the peak level at 60 min (37.04%) which is comparable to that of standard (64.99%), whereas in case of 200 mg/kg body weight dose it was very low which is low central analgesic activity.

The methanolic roots extract produced significant (P < 0.001) 48.13%, 47.42%, 45.74% and 46.06% and 29.08%, 30.14%, 29.80% and 29.66% elongation of tail flicking time at 30, 60, 90 and 120 min respectively after administration of 400 and 200 mg/kg body weight doses respectively. The % elongation of tail flicking time was eventually

Table 3
Central antinociceptive activity of different extracts of *C. assamica* by tail immersion method.

Test samples	Dose (mg/kg)	Reaction times in seconds (Mean \pm SEM) and % MPE				
		Pre-treatment	30 min	60 min	90 min	120 min
Control	–	2.50 \pm 0.13	2.78 \pm 0.12 (2.24%)	3.06 \pm 0.09 (4.48%)	3.44 \pm 0.08 (7.52%)	3.70 \pm 0.04 (9.6%)
Standard	50	2.62 \pm 0.13	6.66 \pm 0.35*** (32.63%)	8.74 \pm 0.38*** (49.43%)	8.24 \pm 0.26*** (45.40%)	7.84 \pm 0.25*** (42.16%)
MS 400	400	2.52 \pm 0.15	5.78 \pm 0.38*** (26.12%)	6.88 \pm 0.29*** (34.94%)	7.64 \pm 0.14*** (41.03%)	6.92 \pm 0.12*** (35.26%)
MS 200	200	2.48 \pm 0.15	4.76 \pm 0.11*** (18.21%)	5.32 \pm 0.15*** (22.68%)	5.72 \pm 0.09*** (25.88%)	5.70 \pm 0.13*** (25.72%)
DFS 400	400	2.76 \pm 0.15	4.96 \pm 0.11*** (17.97%)	5.44 \pm 0.10*** (21.80%)	5.70 \pm 0.11*** (24.02%)	6.00 \pm 0.07*** (26.47%)
DFS 200	200	2.66 \pm 0.16	3.64 \pm 0.20** (7.94%)	4.14 \pm 0.19** (11.99%)	4.54 \pm 0.22*** (15.24%)	4.88 \pm 0.17*** (17.99%)
CFS 400	400	2.46 \pm 0.10	5.04 \pm 0.05*** (20.57%)	5.74 \pm 0.10*** (26.16%)	6.38 \pm 0.17*** (31.26%)	6.76 \pm 0.13*** (34.29%)
CFS 200	200	2.70 \pm 0.16	3.72 \pm 0.10** (8.29%)	4.20 \pm 0.08*** (12.20%)	4.32 \pm 0.10** (13.17%)	4.52 \pm 0.11** (14.80%)
ML 400	400	2.86 \pm 0.09	5.52 \pm 0.12*** (21.91%)	5.98 \pm 0.04*** (25.70%)	6.60 \pm 0.09*** (30.81%)	7.18 \pm 0.07*** (35.58%)
ML 200	200	2.72 \pm 0.24	3.60 \pm 0.11* (7.17%)	4.54 \pm 0.09*** (14.82%)	4.48 \pm 0.07*** (14.33%)	4.34 \pm 0.07 (13.19%)
DFL 400	400	2.38 \pm 0.10	4.48 \pm 0.15*** (16.64%)	4.98 \pm 0.18*** (20.60%)	5.46 \pm 0.19*** (24.41%)	5.30 \pm 0.08*** (23.14%)
DFL 200	200	2.66 \pm 0.18	3.48 \pm 0.04 (6.65%)	4.16 \pm 0.09*** (12.16%)	4.28 \pm 0.09** (13.13%)	4.18 \pm 0.11 (12.32%)
CFL 400	400	2.62 \pm 0.12	4.24 \pm 0.09*** (13.09%)	4.86 \pm 0.12*** (18.09%)	5.26 \pm 0.10*** (21.32%)	5.6 \pm 0.15*** (24.07%)
CFL 200	200	2.68 \pm 0.18	3.30 \pm 0.04 (5.03%)	4.18 \pm 0.09*** (12.18%)	4.06 \pm 0.07* (11.20%)	4.06 \pm 0.15 (11.20%)
MR 400	400	2.70 \pm 0.19	5.36 \pm 0.18*** (21.63%)	5.82 \pm 0.24*** (25.37%)	6.34 \pm 0.19*** (29.59%)	6.86 \pm 0.24*** (33.82%)
MR 200	200	2.62 \pm 0.16	3.92 \pm 0.11*** (10.50%)	4.38 \pm 0.15*** (14.22%)	4.90 \pm 0.15*** (18.42%)	5.26 \pm 0.16*** (21.32%)

Each value represents mean \pm SEM (n = 5). One-way ANOVA followed by Dunnett's *t* test. ****P* < 0.001, ***P* < 0.01, **P* < 0.05 compared with the control. MS = Methanolic stem extract; DFS = Dichloromethane soluble fraction of stem extract; CFS = Chloroform soluble fraction of stem extract; PFS = Pet ether soluble fraction of stem extract; ML = Methanolic leaves extract; DFL = Dichloromethane soluble fraction of leaves extract; CFL = Chloroform soluble fraction of leaves extract; MR = Methanolic roots extract.

Table 4
Percent elongation of latency time after administration of all test samples in tail immersion test.

Test samples	Dose (mg/kg)	% elongation of latency time			
		30 min	60 min	90 min	120 min
Standard	50	58.26	64.99	58.25	52.81
MS 400	400	51.90	55.52	54.97	46.53
MS 200	200	41.60	42.48	39.86	35.09
DFS 400	400	43.95	43.75	39.65	38.33
DFS 200	200	23.63	26.09	24.23	24.18
CFS 400	400	44.84	46.69	46.08	45.27
CFS 200	200	25.27	27.14	20.37	18.14
ML 400	400	49.64	48.83	47.88	48.47
ML 200	200	22.78	32.60	23.21	14.75
DFL 400	400	37.95	38.55	37.00	30.19
DFL 200	200	20.11	26.44	19.63	11.48
CFL 400	400	34.43	37.04	34.60	33.93
CFL 200	200	15.76	26.79	15.27	8.87
MR 400	400	48.13	47.42	45.74	46.06
MR 200	200	29.08	30.14	29.80	29.66

increased at 30 min and then decreased gradually in case of 400 mg/kg body weight dose and attained the peak level at 30 min (48.13%) which is quite nearer to that of standard (58.26%), whereas it was increased with time in case of 200 mg/kg body weight dose and attained the peak level at 60 min (30.14%) which is comparable to that of standard (64.99%). So it can be said that the methanolic extracts and their solvent-soluble fractions showed dose-dependency in their antinociceptive activity.

3.5. Antipyretic activity

The results of the antipyretic activity of methanolic leaves extract were shown in Table 5 and Fig. 1. One hour after the administration of extract at both 400 and 200 mg/kg body weight doses, pyrexia level in mice was remarkably reduced (37.96%, *P* < 0.01 and 34.62%, *P* < 0.01) respectively compared to standard paracetamol (53.21%, *P* < 0.001). After two, three and 4 h of administration, the methanolic leaves extract both at 400 and 200 mg/kg body weight doses significantly reduced pyrexia level (59.62% and 58.33%), (85.58% and 70.37%), (97.12% and 87.04%) respectively compared to standard (68.81%, 82.57% and 93.58%; *P* < 0.001) reduction respectively. In

the case of methanolic leaves extract 400 mg/kg, it was observed even higher than that of the standard after 4 h of administration (Fig. 1). That is the activity was found to be sustainable even after 4 h of the administration.

4. Discussion

This study confirmed the presence of various potential phytochemical constituents such as alkaloid, saponins, flavonoids, steroids, glycosides, terpenoids, tannins, carbohydrates, phenols etc. in different extracts of different parts of this plant. From these, the presence of alkaloids and tannins are specifically responsible for the anti-nociceptive activity as it has been found that tannins [22] and alkaloids [23] exhibit good antinociceptive activity. The intraperitoneal administration of acetic acid induces endogenous pain stimulators, such as prostaglandins, histamine and bradykinin, which stimulate the discomfort locally within the body due to excess pain [24]. Prostanoids, particularly PGE2 and PGF2 α , as well as lipoxigenase products level increased remarkably in the peritoneal fluid during the writhing test [25,26]. So this method is mainly responsible for determining peripherally active analgesics. In the tail immersion test, sensitization of the nociceptors by sensory nerves and the involvement of endogenous substances like prostaglandins are decreased [27].

The anti-nociceptive effect of *C. assamica* methanolic stem extract and its dichloromethane fractions and methanolic roots extract were found to be significant (*P* < 0.0001) respectively in the tail immersion model as well as in the acetic acid induced writhing test in their both doses and so it can be said that these three types of extracts exhibit significant analgesia through both peripheral and central mechanisms. In the case of writhing test, the chloroform soluble fraction of stem extract and the methanolic leaves extract in both doses exhibited significant analgesia through the peripheral mechanism. But in case of tail immersion test, both the chloroform soluble fraction of stem extract and the methanolic leaves extract at their 400 mg/kg body weight dose exhibited significant antinociceptive action through central mechanism but at 200 mg/kg body weight dose they showed lower actions. The dichloromethane and chloroform soluble fractions of leaves extract at their 400 mg/kg body weight dose exhibited significant analgesia through both peripheral and central mechanisms but at their 200 mg/kg body weight dose they showed lower activities. Out of several different modes to select administrative doses for animal models, selection

Table 5
Antipyretic activity of methanolic leaves extract of *C. assamica*.

Groups	Dose (mg/kg)	Rectal temperature °F (Mean ± SEM)					
		Test samples administration					
		Before administration		After administration (% reduction in pyrexia)			
		Normal	After 24h	1h	2h	3h	4h
Control	–	98.36 ± 0.12	100.62 ± 0.17	100.32 ± 0.11 (13.27%)	100.08 ± 0.10 (23.89%)	99.88 ± 0.07 (32.74%)	99.8 ± 0.07 (36.28%)
Standard	150	98.4 ± 0.10	100.58 ± 0.22	99.42 ± 0.16*** (53.21%)	99.08 ± 0.14*** (68.81%)	98.78 ± 0.09*** (82.57%)	98.54 ± 0.07*** (93.58%)
ML 400	400	98.42 ± 0.08	100.50 ± 0.14	99.66 ± 0.10** (37.96%)	99.26 ± 0.02*** (59.62%)	98.72 ± 0.04*** (85.58%)	98.48 ± 0.04*** (97.12%)
ML 200	200	98.32 ± 0.13	100.48 ± 0.19	99.78 ± 0.04** (34.62%)	99.22 ± 0.09*** (58.33%)	98.96 ± 0.05*** (70.37%)	98.60 ± 0.06*** (87.04%)

ML = Methanolic leaves extract of *Cissus assamica*; h = hour. °F = Temperature degree in Fahrenheit scale.

based on acute toxicity-test and LD50 value is our prime choice. According to the theory, prior to intervention, a group of animals is received a gradually increased dose to apprehend a toxic dose which leads to calculate LD50. The effective therapeutic dose was taken as one tenth of the median lethal dose (LD50 > 2.0 g/kg) [28] which is practically justified using a logarithmically linked dose. Additionally, the reference control is a pure single molecule drug which is established as much lower than treatment dose for its effectiveness. On the contrary, the crude extract is usually reported to have relatively lower absorption that is harmonized with it LD50 based relatively higher doses. The polar solvent (e.g., methanol and dichloromethane) extract might be hindered the biosynthesis, release and/or action of the chemical agent like prostaglandins and leukotrienes from cyclo-oxygenase and lipo-oxygenase pathways, respectively and thus exhibiting analgesic property by inhibiting the pain sensation. The presence of alkaloids (polar) may avail this analgesic activity [29]. So we can conclude that this plant parts exhibited analgesic action predominantly in both central and peripheral mechanisms.

Yeast induced or other pathogen-induced fever presents an economical and suitable method for investigating new antipyretic drugs [30]. The presence of proteins in pathogens, yeast in this method is linked to fever via inflammatory reactions [31]. Furthermore, the production of cytokines such as interleukin-1 β (IL-1 β) and IL-6, interferon- α (IFN- α), and tumor necrosis factor- α (TNF- α) and prostaglandins like PGE2 and PGI2 act on the brain and thus increase the body temperature [32–34]. Paracetamol, the antipyretic drug used in this study act through numerous ways by reducing prostaglandins level. In the

management of fever, it acts on cyclooxygenase enzymes and exerts antipyretic message within the brain and thereby stimulates anti-inflammatory signals at injury site [35]. Phytochemical compounds like alkaloids, steroids, flavonoids, saponins etc. were found to be present in the methanolic leaves extract of *C. assamica*. It showed a very potential antipyretic action which sustained for a longer duration. The antipyretic effect of steroids and flavonoids have been proved in various studies [36–39]. Various researches have also revealed the fact that medicinal plants that show antinociceptive property have also found with antipyretic as well as anti-inflammatory activities [40–47]. It may be due to the mechanism for suppression of pain, fever, and inflammation can be correlated as inhibition of inflammatory mediators is occurred in each case. This further indicates the evaluation of this plant for anti-inflammatory potential.

5. Conclusion

The outcomes of the present study indicate us with the fact that the plant extracts have noteworthy antinociceptive and antipyretic activity due to the presence of potentially bioactive compounds in them. Our investigations also showed that the methanol leaves extract exerted significant antipyretic effects in a dose dependent manner. Since this plant parts are used as traditional medicines, the extracts should be discovered scientifically for their phytochemical profiles so that the identification and isolation of active components that are responsible for the exerted pharmacological activities can be possible in future study.

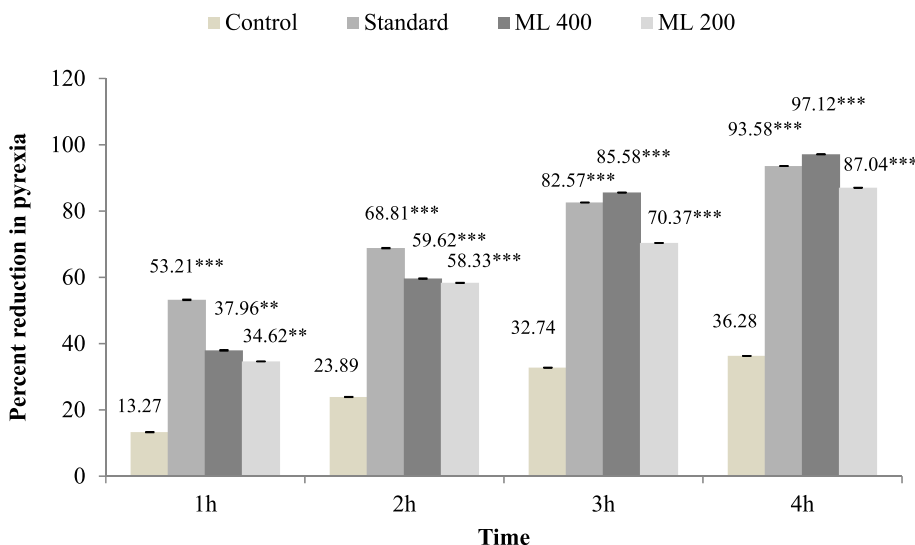


Fig. 1. Periodical percent reduction in pyrexia after administration of doses of methanolic leaves extract of *C. assamica*. Here each value represents mean ± SEM (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001 compared to control, Dunnett's *t*-test after analysis. ML = Methanolic leaves extract of *C. assamica*; 1h, 2h, 3h, 4h, 5h = 1, 2, 3, 4 and 5 h after administration of test samples respectively.

Ethical approval

The study was approved by the Institutional Animal Ethics Committee of the Department of Pharmacy, Faculty of Biological Science of the University of Chittagong, Bangladesh (ref no. ERC/DP/CU/2016/0021). All animal experiments comply with the ARRIVE guidelines and carried out following National Institutes of Health guide for the care and use of Laboratory Animals.

Consent for publication

All authors have agreed to publish all materials belongs to this article.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This work is conducted with the individual funding of all authors.

Author contributions

RAS and TBE together planned and designed the research. RAS arranged the whole facilities for the research. TD conducted the entire laboratory works, AP and MM imparted in study design and interpreted the results putting efforts on statistical analysis with TD, RAS, and TBE. TD, AP, and TBE participated in the manuscript draft and has thoroughly checked and revised the manuscript for necessary changes in format, grammar and English standard. All authors read and agreed on the final version of the manuscript.

Declaration of competing interest

Authors declared that they have no conflict of interest.

Acknowledgements

The authors are indebted to the Department of Pharmacy, Faculty of Biological Science, University of Chittagong, Chittagong-4331, Bangladesh, for providing the facilities to conduct this research work. The authors are also thankful to Bibi Humayra Khanam and Farhana Binta Faiz, Department of Pharmacy, University of Chittagong, Chittagong-4331, Bangladesh for their assistance throughout the research work. The authors are likewise obliged to Mohammed Kamrul Hossain, Professor, Department of Pharmacy, University of Chittagong, Chittagong-4331, Bangladesh for his proper guidance for performing this research.

List of Abbreviations

MS	Methanolic stem extract
DFS	Dichloromethane soluble fraction of stem extract
CFS	Chloroform soluble fraction of stem extract
PFS	Pet ether soluble fraction of stem extract
NFS	N-hexane soluble fraction of stem extract
ML	Methanolic leaves extract
DFL	Dichloromethane soluble fraction of leaves extract
CFL	Chloroform soluble fraction of leaves extract
MR	Methanolic roots extract
SEM	Standard error mean
MPE	Maximal possible effect
h	hour
°F	Temperature degree in Fahrenheit scale

gm	gram
b.w.	body weight

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrep.2019.100715>.

Each value represents mean \pm SEM (n = 5). One-way ANOVA followed by Dunnett's *t* test. ***P < 0.001, **P < 0.01, *P < 0.05 compared with the control. MS = Methanolic stem extract; DFS = Dichloromethane soluble fraction of stem extract; CFS = Chloroform soluble fraction of stem extract; ML = Methanolic leaves extract; DFL = Dichloromethane soluble fraction of leaves extract; CFL = Chloroform soluble fraction of leaves extract; MR = Methanolic roots extract.

References

- [1] S.K. Kulkarni, Handbook of Experimental Pharmacology. Rev vol. 3, Vallabh Prakashan, New Delhi, 1999, pp. 123–125.
- [2] D. Chattopadhyay, G. Arunachalam, L. Ghosh, Antipyretic activity of *Alstonia macrophylla* wall ex A. DC: an ethnomedicine of andaman islands, *J. Pharm. Pharmaceutical Sci.* 8 (2005) 558–564.
- [3] C.B. Spicer, C.D. Breder, The neurologic basis of fever, *N. Engl. J. Med.* 330 (1994) 1880–1886.
- [4] H.M. Said, Development of Herbal Medicine (A Seminar Lecture), Hamdard Foundation, Dhaka, Bangladesh, 1995.
- [5] S.M.K. Rates, Plants as source of drugs, *Toxicol* 39 (2001) 603–613.
- [6] FAO, Trade in Medicinal Plants, Economic and social Department, Food & Agriculture Organization of the United Nations, Rome, 2004, pp. 2–3.
- [7] M. Yusuf, J. Begum, M.N. Hoque, J.U. Chowdhury, Medicinal Plants of Bangladesh (Revised and Enlarged), Bangladesh Council of Scientific and Industrial Research Laboratories, Chittagong, Bangladesh, 2009, p. 794.
- [8] Y.H. Xie, P. Deng, Y.Q. Zhang, W.S. Yu, Studies on the chemical constituents from *Cissus assamica*, *Zhong Yao Cai* 32 (2) (2009) 210–213.
- [9] A. Ghani, Medicinal Plants of Bangladesh with Chemical Constituents and Uses vol. 5, Asiatic society of Bangladesh, Dhaka: Bangladesh, 2003.
- [10] P. Tiwari, B. Kumar, M. Kaur, G. Kaur, H. Kaur, Phytochemical screening and Extraction: a review, *Int. Pharm. Sci.* 1 (1) (2011) 98–106.
- [11] R. Koster, M. Anderson, E.J. Beer, Acetic acid for analgesic screening. *Fed. SAVE Proc.* 18 (1959) 412.
- [12] H.G. Vogel, W.H. Vogel, Drug Discovery and Evaluation-Pharmacological Assays vols. 370–1, Springer-Verlag, New York, 1997, p. 402.
- [13] A.S. Apu, F. Hossain, F. Rizwan, S.H. Bhuyan, M. Matin, A. Jamaluddin, Study of pharmacological activities of methanol extract of *Jatropha gossypifolia* fruits, *J. Basic Clin. Pharm.* 4 (2013) 20e24.
- [14] P.A. Janssen, C.J. Niemegeers, J.G. Dony, The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats, *Arzneimittelforschung* 13 (1963) 502–507.
- [15] O.O. Adeyemi, O.O. Adegun, O.K. Yemitan, Analgesic activity of the aqueous root extracts of *Lecaniodiscus cupanioides*, *West Afr. J. Pharmacol. Drug Res.* 19 (2005) 42–46.
- [16] G. Woolfe, A.D. MacDonald, The evaluation of analgesic action pethidine hydrochloride, *J. Pharmacol. Exp. Ther.* 80 (1994) 300.
- [17] P. Malairajan, G. Gopalakrishnan, S. Narasimhan, K.J.K. Veni, Analgesic activity of some Indian medicinal plants, *J. Ethnopharmacol.* 106 (3) (2006) 425–428.
- [18] S.H. Fan, N.A. Ali, D.F. Basri, Evaluation of analgesic activity of the methanol extract from the galls of *Quercus infectoria* (Olivier) in rats. *Evid Based Complement. Altern. Med.* (2014) 1–6. Article ID 976764.
- [19] R.K. Kumawat, S. Kumar, S. Sharma, Evaluation of analgesic activity of various extracts of *Sida tiagii* Bhandari, *Acta Pol. Pharm.* 69 (2012) 1103–1109.
- [20] M.S. Al-Ghamdi, The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*, *J. Ethnopharmacol.* 76 (2001) 45–48.
- [21] E. Woode, R.A. Poku, G.K. Ainooson, E. Boakye-Gyasi, W.K.M. Abotsi, T.L. Mensah, A.K. Amoh-Barimah, An evaluation of the anti-inflammatory, antipyretic and antinociceptive effects of *Ficus exasperate* (Vahl) leaf extract, *J. Pharmacol. Toxicol.* 4 (2009) 138–151.
- [22] M.R. Vanu, S. Palanivelu, S. Panchanatham, Immunomodulatory and anti-inflammatory effects of *Semecarpus anacardium* Linn. nut milk extract in experimental inflammatory conditions, *Biol. Pharm. Bull.* 29 (2006) 693–700.
- [23] F.I. Uche, J.S. Aprioku, The phytochemical constituents, analgesic and anti-inflammatory effects of methanol extract of *Jatropha curcas* leaves in mice and Wister albino rats, *J. Appl. Sci. Environ. Manag.* 12 (4) (2008) 99–102.
- [24] M.D. Obispo, R.H.V. Mourao, E.M. Franzotti, K.B.R. Bomfim, M.F. Arrigoni-Blank, M.P.N. Moreno, Antinociceptive and anti-inflammatory effects of the aqueous extract of *Hyptis pectinata* leaves in experimental animals, *J. Ethnopharmacol.* 76 (1) (2001) 81–86.
- [25] R. Deraedt, S. Jougney, F. Delevalce, M. Falhout, Release of prostaglandin E and F in an alogenic reaction and its inhibition, *Eur. J. Pharmacol.* 61 (1980) 17–24.
- [26] A.K. Dhara, V. Suba, T. Sen, S. Pal, A.K. Chaudhuri, Preliminary studies on the anti-

- inflammatory and analgesic activity of methanolic fraction of the root of *Tragia involucrate*, *J. Ethnopharmacol.* 72 (2000) 265–268.
- [27] R.S. Bachlav, V.S. Gulecha, C.D. Upasani, Analgesic and anti-inflammatory activity of *Argyrea speciosa* roots, *Indian J. Pharmacol.* 41 (4) (2009) 158–161.
- [28] A. Zaoui, Y. Cherrah, N. Mahassini, K. Alaoui, H. Amarouch, M. Hassar, Acute and chronic toxicity of *Nigella sativa* fixed oil, *Phytomedicine* 9 (2002) 69–74.
- [29] F. Uche, J. Aprioku, The phytochemical constituents, analgesic and anti-inflammatory effects of methanol extract of *Jatropha curcas* leaves in Mice and Wister albino rats, *Appl. Sci. Environ. Manage.* 12 (4) (2008) 99–102.
- [30] J. Tomazetti, D.S. Avila, A.P. Ferreira, Baker yeast induced fever in young rats: characterization and validation of an animal model for antipyretics screening, *J. Neurosci. Methods* 147 (1) (2005) 29–35.
- [31] J.S.M. Pasin, A.P.O. Ferreira, A.L.L. Saraiva, Diacerein decreases TNF- α and IL-1 β levels in peritoneal fluid and prevents Baker's yeast-induced fever in young rats, *Inflamm. Res.* 59 (3) (2010) 189–196.
- [32] G.N. Luheshi, et al., Cytokines and fever: mechanisms and sites of action, *Ann. NY Acad. Sci.* 856 (1998) 83–89.
- [33] C.B. Saper, C.D. Breder, Theneurologic basis of fever, *N. Engl. J. Med.* 330 (26) (1994) 1880–1886.
- [34] W.L. Veale, K.E. Cooper, Q.J. Pittman, P. Ramwell (Ed.), Role of Prostaglandins in Fever and Temperature Regulation in the Prostaglandins, vols. 145–167, Springer, New York, NY, USA, 1977.
- [35] D.M. Aronoff, E.G. Neilson, Antipyretics: mechanisms of action and clinical use in fever suppression, *Am. J. Med.* 111 (4) (2001) 304–315.
- [36] H.P. Kim, K.H. Son, H.W. Chang, S.S. Kang, Antiinflammatory plant flavonoids and cellular action mechanisms, *J. Pharmacol. Sci.* 96 (2004) 229–245.
- [37] J. Niazi, P. Singh, Y. Bansal, R.K. Goel, Anti-inflammatory, analgesic and antipyretic activity of aqueous extract of fresh leaves of *Coccinia indica*, *Inflammopharmacology* 17 (2009) 239–244.
- [38] J. Niazi, V. Gupta, P. Chakarborty, P. Kumar, Antiinflammatory and antipyretic activity of *Aleuritis moluccana* leaves, *Asian J. Pharmaceut. Clin. Res.* 3 (1) (2010) 35–37.
- [39] V. Tiwari, A. Tiwari, V. Madhavan, Preliminary phytochemical analysis, HPTLC studies and antipyretic activity of alcohol and aqueous extract of *Helicteres isora* L. Root, *Int. J. Pharm. Pharm. Sci.* 2 (2) (2010) 74–79.
- [40] T. Afsar, M.R. Khan, S. Razak, S. Ullah, B. Mirza, Antipyretic, anti-inflammatory and analgesic activity of *Acacia hydaspica* R. Parker and its phytochemical analysis, *BMC Complement Altern. Med.* 15 (2015) 136.
- [41] A. Rauf, G. Uddin, B.S. Siddiqui, N. Muhammad, H. Khan, Antipyretic and antinociceptive activity of *Diospyros lotus* L. in animals, *Asian Pac. J. Trop. Biomed.* 4 (1) (2014) S382–S386.
- [42] S. Ahmed, A. Rakib, M.A. Islam, B.H. Khanam, F.B. Faiz, A. Paul, M.N.U. Chy, N.M.M.A. Bhuiya, M.M.N. Uddin, S.M.A. Ullah, M.A. Rahman, T.B. Emran, In vivo and in vitro pharmacological activities of *Tacca integrifolia* rhizome and investigation of possible lead compounds against breast cancer through *in silico* approaches. *Clin. Phytoscience.* 5 (36) (2019) 1–13, <https://doi.org/10.1186/s40816-019-0127-x>.
- [43] M.Z. Uddin, M.S. Rana, S. Hossain, E. Dutta, S. Ferdous, M. Dutta, T.B. Emran, In vivo neuroprotective, antinociceptive, anti-inflammatory potential in Swiss albino mice and in vitro antioxidant and clot lysis activities of fractionated *Holigarna longifolia* Roxb. bark extract, *J. Complement. Integ. Med.* 17 (2) (2019) 1–9, <https://doi.org/10.1515/jcim-2019-0102>.
- [44] Z. Al Mahmud, T.B. Emran, N. Qais, S.C. Bachar, M. Sarker, M.M.N. Uddin, Evaluation of analgesic, anti-inflammatory, thrombolytic and hepatoprotective activities of roots of *Premna esculenta* (Roxb.), *J. Basic Clin. Physiol. Pharmacol.* 27 (1) (2016) 63–70, <https://doi.org/10.1515/jbcp-2015-0056>.
- [45] Z. Al Mahmud, N. Qais, S.C. Bachar, C.M. Hasan, T.B. Emran, M.M.N. Uddin, Phytochemical investigations and antioxidant potential of leaf of *Leea macrophylla* (Roxb.), *BMC Res. Notes.* 10 (24) (2017) 1–9, <https://doi.org/10.1186/s13104-017-2503-2>.
- [46] M.A.M. Momin, S.M.F. Bellah, S.M.R. Rahman, A.A. Rahman, G.M.M. Murshid, T.B. Emran, Phytopharmacological evaluation of ethanol extract of *Sida cordifolia* L. roots, *Asian Pacific J. Trop. Biomed.* 4 (1) (2014) 18–24, [https://doi.org/10.1016/S2221-1691\(14\)60202](https://doi.org/10.1016/S2221-1691(14)60202).
- [47] T.B. Emran, M.A. Rahman, Sedative, anxiolytic and analgesic effects of *Urena sinuata* L. leaf extract in animal models, *Int. Food Res. J.* 21 (5) (2014) 2069–2075.