academicJournals

Vol. 5(6), pp. 106-113, June, 2013
DOI: 10.5897/JPP2013.0268
ISSN 2141-2502 ©2013 Academic Journals
http://www.academicjournals.org/JPP

Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Evaluation of analgesic, antipyretic and anti-inflammatory effects of methanol extract of traditional herbal medicine using rodents

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Accepted 24 April, 2013

The analgesic, antipyretic and anti-inflammatory effects of methanol extract of botanical medicine prepared by taking equal amounts of dried stems of *Tinospora cordifolia* Willd, fruits of *Emblica officinalis* Gaertn and rhizomes of *Cyperus rotundus* Linn were evaluated using standard methods. These botanicals have been traditionally used in Indian system of medicine for treatment of fever, joint pain, body ache and muscular pain in the form of single or combined drug therapy. After acute toxicity studies, anti-inflammatory effect was assessed using carrageen induced paw oedema test and antipyretic effect using yeast induced pyrexia method. Tail immersion, hot plate and writhings test were used for determining the analgesic properties. Phytochemical analysis revealed the presence of polyphenolic flavonoids, tannin and saponins. Significant anti-inflammatory, antipyretic and analgesic properties were noticed in dose dependant manner after methanolic extract administration, especially at 500 mg/kg dose. These test drug activities were sustained and comparable to the standard drugs while exhibiting no acute toxicity. The findings indicate that the methanolic extract possesses significantly high anti-inflammatory, antipyretic and analgesic properties without any acute toxicity possibly due to the presence of flavonoids.

Key words: Analgesic, antipyretic, anti-inflammatory, toxicity, botanical medicine.

INTRODUCTION

The traditional Indian system of Ayurvedic medicine has mentioned a number of therapeutic cures for common ailments using specific plant parts or their combinations which are relatively free from the side-effects commonly associated with the modern Allopathic system of medicine. The botanical medicine under examination in this study consists of the methanolic extract of equal amounts of dried stems of *Tinospora cordifolia* Willd, fruits of *Emblica officinalis* Gaertn and rhizomes of *Cyperus rotundus* Linn. This combined formulation has been used in the Indian system of medicine (Ayurveda) for the treatment of fever, joint pain, body ache, muscular pain and as a rejuvenator for thousands of years, and has been mentioned for its above mentioned properties in the ancient traditional textbook Charak Samhita (Shastri, 2003).

T. cordifolia (family Menispermaceae) is a glabrous clim-bing plant which is mostly found in deciduous and dry forest regions of India. The succulent stem is creamy white to grey in color, with deep clefts spotted with lenticels

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often giving out aerial roots. The principal constituents found in its stem are tinosporin, tinocordiside, tinocordifolioside, cordioside and alkaloids like berberine, palmatine (Sharma et al., 2005). *E. officinalis* Gaertn also known locally as “Amalaki” is a small or medium sized deciduous tree belonging to Euphorbiaceae family found throughout India. Dried fruit of the plant is brown to blackish brown in colour with characteristic odour and sour and astringent taste. Its fruits are a rich source of Vitamin C, tannin, ellagic acid and gallic acid (Rao and Siddiqui, 1964). *C. rotundus* Linn belonging to the family Cyperaceae is a perennial weed indigenous to India but is now spread in tropical, subtropical and temperate regions. The rhizomes known as “Mustak” are bluntly conical and vary in size and thickness, crowned with the remains of stem and leaves forming a scaly covering, dark brown or black externally, creamish-yellow internally and have pleasant odour. The rhizome contains polyphenols like cyperone, cyperenone and cyperene and carbohydrates like D-glucose and D-fructose. The rhizome is reported to possess analgesic, anti-inflammatory and antipyretic activities (Guldur et al., 2010; Nagulendran, 2007).

The present study undertakes the phytochemical and biological evaluation of this botanical medicine. Assessment of the analgesic, antipyretic and anti-inflammatory pharmacological activities of the methanolic extract in case of rodents was carried out and the results obtained were compared with the standard non-steroidal anti-inflammatory synthetic drugs. The objective is to validate the efficacy of this traditional herbal non-toxic, antipyretic, analgesic and anti-inflammatory drug which has been mentioned in Ayurvedic system of medicine.

MATERIALS AND METHODS

The pharmacognostical and experimental studies were done in the laboratory and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) registered animal house facility of Dravyaguna Department of the Institute of Post Graduate Ayurvedic Education and Research, Kolkata.

Plant materials and extraction of botanical medicine

The dried plant parts of research drug were collected from the authentic medicinal herb supplier of the Pharmacy department of this institute and authenticated by the Department of Botany of the Botanical Survey of India at Shibpur, Howrah (Ref No- BSI/CNH/AD/Tech/2010, Date- 21.07.2010). All documents and samples have been kept in the museum of this department. The analytical grade chemicals for phytochemical screening along with standard reagents were purchased from M/s Merck Specialities Pvt. Ltd, Mumbai, India.

Animals

All the experimental studies were carried out under suitable conditions in the animal house and experimentation facility of the Institute of Post Graduate Ayurvedic Education and Research (IPGAER), Kolkata (registered under CPCSEA Reg. No- 1180/ac/08/CPCSEA) after taking all required permissions from the Institutional Animal Ethical Committee. The rats and mice required for experiments were procured from M/s Ghosh Scientific, Kolkata. The animals were fasted overnight prior to experiments but allowed free access to water. The anti-inflammatory and antipyretic experiments were performed on adult Wistar rats of either sex (1 month old and weighing 80 to 120 g). Swiss albino mice weighing 20 to 30 g were used for analgesic and acute toxicity study. They were fed with standard pellet diet and water *ad libitum* in poly-propylene cages and maintained under environmentally controlled room temperature of approximately 25°C.

Extraction of botanical medicine

The 40 mesh sized sun dried powdered fruits of *E. officinalis*, stems of *T. cordifolia* and rhizomes of *C. rotundus* were mixed in equal proportion to prepare the research drug after removing all extraneous matters such as dirt, foreign particles and adulterants. The research drug was extracted in methanol using a Soxhlet apparatus for 36 h. The obtained extract was filtered, concentrated in a rotary evaporator and finally stored in refrigerator for further analysis (Trease and Evans, 2009). The methanolic extract of the test drug was used throughout the experimental and chemical analysis.

Pharmacognostical study of crude drug powder

The macroscopic and microscopic study of the powder of test drug was performed according to the standard procedures. The final crude drug powder was mounted in glycerine, observed and photographed under the optical microscope (40×) of Dewinter, Italy.

Physiochemical analysis

The analysis of the physiochemical parameters such as moisture content, extractive value, acid insoluble ash, water soluble ash and total ash content of the test drug powder was performed according to the standard steps described in the Ayurvedic Pharmacopoeia, Government of India (2001).

Estimation of total phenolic content

The total phenolic content of the drug was determined by Folin Ciocalteu method using gallic acid as standard compound at different concentrations (100 to 500 µg/ml). The assessed total phenol values were expressed in terms of gallic acid equivalent as mg/g of dry mass.

Phytochemical screening

Preliminary phytochemical screening of the different constituents like alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, fats and oils, proteins and amino acids was performed following standard procedures.

Estimation of acute toxicity

Organisation for Economic Co-operation and Development (OECD) guideline # 423 was followed for carrying out acute oral toxicity studies (OECD, 2001). The animals of both sexes were selected by sampling technique and were divided into 6 groups of 3 animals

each. A single oral dose of the extract starting at 100 mg/kg and progressively moving from 300, 500, 700, 1000 mg/kg up to 1200 mg/kg was administered. All the animals were observed for appearance of toxic symptoms including muscle spasm, loss of righting reflex, tremors, behavioural changes, locomotion, convulsions and mortality for 24 h. Long term supervision was continued for a period of 14 days to observe any occurrence of toxic symptoms and mortality.

Evaluation of analgesic activity

Assessment of central analgesic effect

Hot plate method: The central analgesic activity of the methanol extract of test drug against thermal stimulus was evaluated in mice following hot plate method as well as tail immersion method (Ghosh, 2008; Hajare et al., 2000). Morphine sulphate (2.5 mg/kg i.m) was used as the standard drug. The standard group (B) was given intramuscular injection of 2.5 mg/kg morphine while the control group (A) was orally administered 10 ml/kg saline solution. Treatment groups C and D were orally administered 300 and 500 mg/kg of test drug, respectively one hour before applying the thermal stimulus, which was maintained at 55 ± 0.2°C. The delay in hind paw licking by each animal was recorded in seconds as response after 10, 30 and 60 min of drug administration. Maximum reaction time of observation was kept at 60 s throughout to avoid tissue damage (Ibironke and Ajiboye, 2007).

Tail immersion method: The tail immersion method was used to assess the central analgesic effect of methanol extract in different dosages (Ghosh, 2010; Hajare et al., 2000; Veerappan et al., 2005). The standard group B was injected with 2.5 mg/kg i.m of morphine sulphate which was used as the standard drug while the control group was given 10 ml/kg of saline. The groups C and D were orally administered 300 and 500 mg/kg of the test drug, respectively 1 h before applying thermal stimulus by placing the tail 5 cm in the glass beaker containing water temperature maintained at 55 ± 0.2°C. The delay in withdrawing of the tail from the glass beaker was recorded in seconds as responses after 10, 30 and 60 min of drug administration. The maximum time of observation would be about 60 s throughout to avoid any tissue damage.

The assessment of peripheral analgesic effect

The peripheral analgesic activity of the methanolic extract of test drug was assessed in acetic acid induced writhing experiments using mice. The standard procedure prescribed by Veerappan et al. (2005) was used for observing the abdominal constriction writhings resulting from intraperitoneal injection of acetic acid (10 ml/kg of 0.6% v/v glacial acetic acid solution in water) (Gupta et al., 2008). Saline (10 ml/kg) was orally administered to group A (control group) whereas standard aspirin (100 mg/kg) was prescribed for group B and 300 and 500 mg/kg test drug extract was orally administered to groups C and D, respectively. Acetic acid solution was then administered to each animal after 30 min and the number of writhings counted for the next 15 min.

Evaluation of antipyretic activity

The estimation of antipyretic efficacy of methanolic extract was carried out using brewer's yeast induced pyrexia method. Fever was induced by means of subcutaneously injecting 10.0 ml/kg of a 20% w/v suspension of Brewer's yeast in normal saline. Only animals whose rectal temperature increased by at least 1.0°C after 18 h of this yeast injection were included for the study. The normal rectal temperature of each animal was measured by using a flexible tail thermostat probe coated with lubricant, and temperature was recorded using a digital IMCORP Telethermometer. The experimental animals were randomly divided into four groups containing six animals in each group. The control group (A) was orally administered 0.5 ml saline while the standard group (B) was given 100 mg/kg aspirin and groups C and D were prescribed 300 and 500 mg/kg of methanol extract of test drug, respectively. The rectal temperature was recorded at time intervals of 1, 2, 3, 4 and 5 h after drug administration.

Anti-inflammatory studies (carrageenan-induced paw oedema in rats)

During anti-inflammatory studies, paw oedema was induced by injecting 0.1 ml of 1% (w/v) carrageenan suspension into the sub planter region of the right hind paw of the rats (Mahat and Patil, 2007). The control group (A) was orally administered saline (10 ml/kg) while the standard group (B) was given indomethacin (5 mg/kg), and groups C and D were given 300 and 500 mg/kg of the test drug extract 1 h before carrageenan injection. The measurement of paw oedema was carried out by displacement technique using plethysmometer to find out the circumference of paw oedema immediately before and after 1, 2, 3 and 4 h following the carrageenan injection. The inhibitory activity was calculated according to the formula:

$$\% \text{ Inhibition} = \frac{(C_t - C_o) \text{ control} - (C_t - C_o) \text{ treated}}{(C_t - C_o) \text{ control}} \times 100$$

Where C_t = paw circumference at time t, C_o = paw circumference before carrageenan injection, $(C_t - C_o)$ = oedema or change in paw size after time t.

Statistical analysis

The differences in individual data values for different parameters obtained during the above mentioned experiments were examined by two-way analysis of variance using ANOVA test. The level of significance was fixed between $p < 0.05$ to $p < 0.01$ (Krishnaswami and Ranganatham, 2011).

RESULTS

Physiochemical analysis

The results of the physiochemical analysis are as given in Table 1.

Estimation of acute toxicity

The animals tested in acute toxicity tests up to the dose of 1200 mg/kg, showed no significant toxic symptoms like sedation, convulsion, diarrhoea, irritation, etc. and no signs of behavioural changes. No mortality was reported up to 24 h and even later during the subsequent 14 days at 1200 mg/kg dose.

Table 1. Results of the physiochemical analysis.

Parameter	Value (% w/w)
Yield of dried methanol extract	3.78
Moisture content	8.54
Total ash content	6.08
Acid insoluble ash	2.11
Water soluble ash content	3.79
Total phenol content	25.86 mg/g of dry mass

Evaluation of analgesic activity

Assessment of central analgesic effect

Hot plate method: During the hot plate method, after giving thermal stimulus, reaction time increased significantly up to 60 min in case of the 300 mg/kg drug group (increasing from 3.22 to 5.38 s) and 500 mg/kg test drug group (increasing from 3.15 to 5.75 s) when compared with the control group (where it actually decreased from 2.89 to 2.86 s). The test drug at the dose of 500 mg/kg showed similar type of pattern up to 90 min (reaction time increasing from 3.15 to 6.65 s) but slightly lower central analgesic effect when compared with the standard drug morphine sulphate (where the reaction time increased from 3.05 to 6.88 s) (Table 2 and Figure 1). The results of the hot plate method showed that after oral administration of drug and giving of thermal stimulus to the animals, the percentage increase in reaction time up to 60 min was 115.73% in case of standard group, 67.08% in case of 300 mg/kg test drug group and 82.53% in case of 500 mg/kg group. In comparison to this, the control group actually showed a decrease of 1.04% in reaction time over the same time period. The test drug thus exhibited significant central analgesic effect at both the doses of 300 and 500 mg/kg when compared with the control group. However, the results of test drug assessed in terms of the average reaction time at the dose of 500 mg/kg (82.53% increase) showed less central analgesic effect when compared to the standard drug morphine (115.73% increase).

Tail immersion method: The results of the tail immersion method showed significant increase in reaction time up to 60 min after giving thermal stimulus in case of both the 300 mg/kg methanolic extract of test drug group (increasing from 2.62 to 4.62 s) and the 500 mg/kg test drug group (increasing from 3.01 to 5.70 s) when compared with the control group (where it increased from 2.24 to 2.45 s). The 500 mg/kg test drug group showed similar type of pattern up to 90 min (reaction time increasing from 2.62 to 4.15 s), however its overall central analgesic effect was a little lower when compared with the standard drug morphine where the reaction time increased from 2.89 to 5.87 s during this period (Table 3). The percentage increase in reaction time after oral administration of the drug and giving of thermal stimulus to the animals in case of tail immersion method up to 60 min was 126.9% in case of the standard group, 76.33% in case of the 300 mg/kg test drug group and 89.36% in case of the 500 mg/kg group (Figure 2). During the same period, the control group showed only 9.37% increase in reaction time, indicating significant impact of the test drug and standard drug during the experiment. The results indicated that the test drug exhibited significant central analgesic effect at both the doses of 300 and 500 mg/kg as compared to the control group. However, the overall impact of the test drug assessed in terms of the average reaction time at the dose of 500 mg/kg (89.36% increase) showed lower central analgesic effect as compared with the standard drug morphine (126.9% increase).

Assessment of peripheral analgesic effect (acetic acid induced writhing analysis)

The peripheral analgesic effect was evaluated on the basis of the average number of abdominal constrictions indicated by the extension of hind paw of animals during the writhings test. The observed inhibition in writhings as a result of administration of the test drug up to 15 min was significantly higher ($p < 0.05$) at the dose of 300 mg/kg (43.31%) as well as at 500 mg/kg (52.08%) when its activity was compared with the control group (Table 4). Comparing the performance of the test drug with the standard drug, the observed peripheral analgesic effect was slightly less at the test drug dose of 500 mg/kg as indicated by 52.08% inhibition in writhings as compared to the standard drug aspirin which resulted in 58.75% inhibition (Figure 3).

Antipyretic effect on yeast induced pyrexia

The antipyretic effect of the methanolic extract of test drug on yeast induced pyrexia has been shown in Table 4. The experimental rats showed a mean increase of about 1.58°C in rectal temperature after 18 h of yeast injection. The test drug extract at both the doses of 300 and 500 mg/kg showed significant antipyretic effect. The antipyretic effect of the extract at the dose of 500 mg/kg is highly significant ($p < 0.05$) and comparable to that of the standard drug aspirin when compared to the control group. There had been a sharp decrease in elevated rectal temperature after 1 h of drug administration which was further followed by a gradual trend of decrease in a dose dependent manner up to 5 h of oral administration of the drug sample in each group (Table 5).

The percentage reduction of rectal temperature after yeast injection in the individual groups after 4 h was 0.59% in the control group, 1.99% in case of the standard drug, 1.23% in the 300 mg/kg test drug and 1.94% in the 500 mg/kg test drug group after oral administration of all

Table 2. Average reaction time using Hot plate method.

Group	0 min	30 min	60 min	90 min	% increase in reaction time after 60 min
Control	2.89±0.039	2.94±0.086	2.86±0.035	2.85±0.121	-1.04
Standard	3.05±0.054	5.98±0.061	6.58±0.040	6.88±0.0 81	115.73
Methanolic extract (300 mg/kg)	3.22±0.054	4.42±0.083	5.38±0.071	6.18±0.029	67.08
Methanolic extract (500 mg/kg)	3.15±0.033	5.01±0.040	5.75±0.050	6.65±0.034	82.53

Results are presented as mean ± standard error of mean (SEM). $p < 0.05$ compared to control n = 6

Table 3: Average reaction time during tail immersion method.

Group	0 min	30 min	60 min	90 min	% increase in reaction time after 60 min
Control	2.24±0.046	2.56±0.073	2.45±0.082	2.30±0.053	9.37
Standard	2.89±0.064	5.99±0.150	6.56±0.095	5.87±0.079	126.9
Methanolic extract (300 mg/kg)	2.62±0.048	4.00±0.037	4.62±0.031	4.15±0.056	76.33
Methanolic extract (500 mg/kg)	3.01±0.033	5.44±0.042	5.70±0.042	5.65±0.040	89.36

Results are presented as mean ± standard error of mean (SEM). $p < 0.05$ compared to control n = 6

Table 4. Analgesic effect during writhing method.

Group	Average number of writhings/15 min	% Inhibition
Control	50.38±1.4 5	0.00
Standard	20.78±0.76	58.75
Methanolic extract (300 mg/kg)	28.56±1.30	43.31
Methanolic extract (500 mg/kg)	24.14±0.91	52.08

Results are presented as mean ± standard error of mean (SEM). $p < 0.05$ compared to control n = 6.

Table 5. Antipyretic effect of methanol extract in comparison to aspirin.

Parameter	Initial rectal temperature in ℃	Rectal temperature in ℃ after 18 h of yeast Injection (Mean±SEM)						% reduction of rectal temperature after 4 h
		0 h	1 h	2 h	3 h	4 h	5 h	
Control	36.28±0.03	37.6±0.06	37.57±0.02	37.55±0.04	37.49±0.03	37.38±0.04	37.35±0.04	0.59
Standard	36.89±0.05	38.07±0.04	37.56±0.03	37.5±0.03	37.43±0.06	37.31±0.02	37.33±0.03	1.99
Methanolic extract (300 mg/kg)	36.31±0.04	37.4±0.02	37.03±0.04	36.99±0.03	36.95±0.04	36.94±0.05	36.96±0.04	1.23
Methanolic extract (500 mg/kg)	36.36±0.04	37.2±0.03	36.59±0.05	36.57±0.02	36.51±0.03	36.48±0.04	36.5±0.02	1.94

Results are presented as mean±SEM. $p < 0.05$ compared to control n = 6.

Table 6. Anti-inflammatory effect during carrageen–induced paw oedema test.

Treatment group	0 h	1 h	2 h	3 h	4 h	% inhibition after 4 h
Control (10 ml/kg)	0.48±0.08	1.44±0.06	2.53±0.07	2.85±0.09	2.86±0.05	-
Indomethacin (5 mg/kg)	0.45±0.05	0.86±0.08	0.74±0.06	0.66±0.07	0.60±0.07	79.02
Methanolic extract (300 mg/kg)	0.42±0.04	0.94±0.07	0.89±0.08	0.84±0.05	0.81±0.08	71.68
Methanolic extract (500 mg/kg)	0.44±0.07	0.93±0.04	0.88±0.05	0.79±0.07	0.73±0.05	74.48

Results are presented as Mean±SEM. $p < 0.05$ compared to control n = 6.

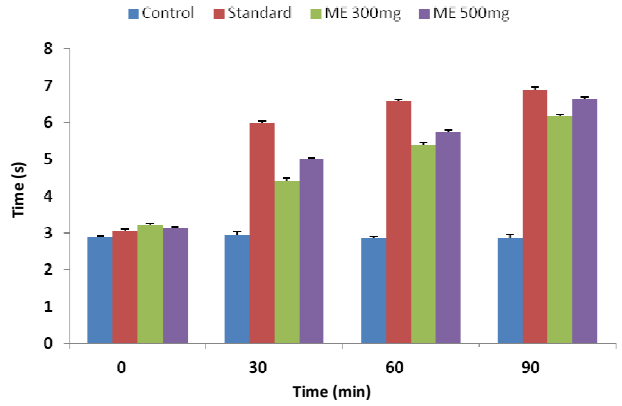

Figure 1. Reaction time during hot plate method.

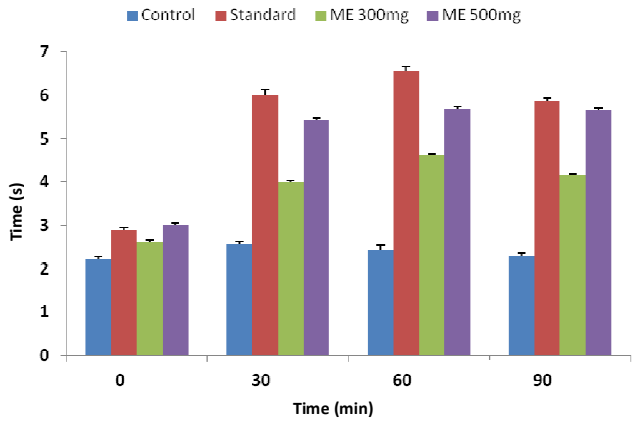

Figure 2. Central analgesic effect during tail immersion method.

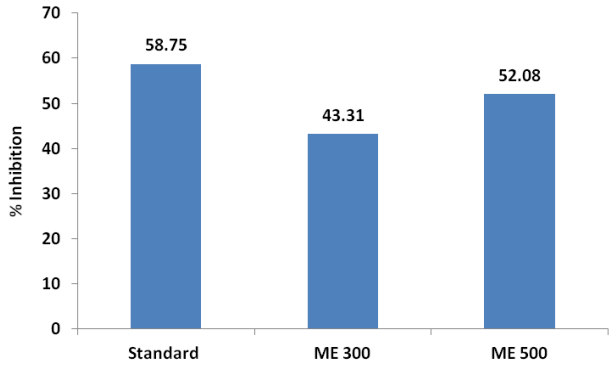

Figure 3. Peripheral analgesic effect in writhing method.

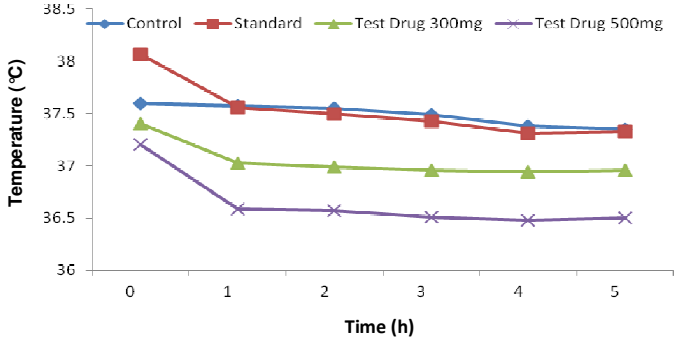

Figure 4. Rectal temperature of rats during yeast induced pyrexia test.

drug samples. The antipyretic effect on the basis of percentage reduction of rectal temperature after 4 h showed quite highly significant results of 500 mg/kg test drug as compared to the standard drug aspirin. The test drug also exhibited more sustained and persistent anti-pyretic effect lasting up to 5 h during this test (Figure 4).

Anti-inflammatory studies (carrageenan-induced paw oedema)

The circumference of paw oedema in rats induced by the carrageen agent to evaluate the acute anti-inflammatory effect at 300 and 500 mg/kg dose showed significant decrease in inflammation of 71.68 and 74.48%, respectively ($p < 0.05$) up to 4 h as compared to the control group (Table 6). The percentage inhibition of the acute inflammation in the paw of rats was found to be comparable but a little lower in the 500 mg/kg test drug dosage group (74.48% inhibition) compared to the standard drug indomethacine (79.02% inhibition) after 4 h of treatment (Figure 5).

DISCUSSION

Inflammation is defined as a biological process

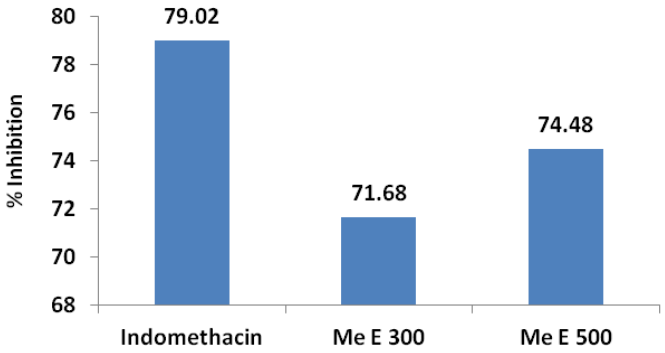

Figure 5. Anti-inflammatory effect during carrageen–induced paw oedema test.

characterized by redness, oedema, fever and pain, and can result in locally increased production of free radicals by inflammatory enzymes, as well as the release of inflammatory mediators that promote cell proliferation and angiogenesis and inhibit apoptosis. Although the currently used steroidal and non-steroidal anti-inflammatory drugs treat acute inflammatory disorders, these conventional drugs have not been successful in curing chronic inflammatory disorders such as rheumatoid arthritis. Moreover, usage of many of such drugs has been associated with adverse impact upon the hepatic and renal functions (Kim et al., 2004; Rajnarayana et al., 2006; Warden, 2010).

The preliminary chemical analysis of this methanolic extract following standard method indicates the presence of high concentration of phenolic compounds such as flavonoids, tannins and carbohydrates. Flavonoids are a large family of compounds synthesized by plants that have a common chemical structure. The flavonoidic phenolic compounds have been known to exhibit anti-inflammatory, antioxidant and metal-chelating properties (Rajnarayana et al., 2006; Pan et al., 2010). Inflammation can result in the locally increased production of free radicals by inflammatory enzymes, as well as the release of inflammatory mediators that promote cell proliferation and angiogenesis and inhibit apoptosis (Gupta et al., 2012).

The hot plate and tail immersion methods indicated that the central analgesic effect of the methanol extract of test drug was significant and dose dependent as revealed by the increased reaction time after giving thermal stimulus to the mice. The latency in reaction time continued up to 90 min after the drug administration in both 300 and 500 mg/kg extracts revealing sustained and pronounced central analgesic effect when compared with the control group. However, its overall central analgesic effect was a little lower when compared with the standard drug morphine over different time periods; the higher test drug dosage resulting in a more pronounced effect with a similar pattern of results.

The assessment of peripheral analgesic effect of the test drug exhibited significant percentage inhibition in the writhings which were induced by acetic acid in the mice at both the 300 and 500 mg/kg doses of the methanolic extract when compared with the control group. The percentage inhibition of writhings at the higher dose of 500 mg/kg indicated the pronounced peripheral analgesic effect in the context of visceral pain which was comparable to the standard pure drug morphine within 15 min of test. The overall central analgesic effect of the test drug even at higher dose was a little lower than the standard drug morphine. The significant analgesic effect at the higher dose was attributed to the presence of high concentration of flavonoidic compounds which inhibited the synthesis, release or receptor responses in prostaglandin mediated effects (Gupta et al., 2008; Pan et al., 2010; Gupta et al., 2012).

During antipyretic tests, the percentage reduction in rectal temperatures after yeast injection up to 4 h was quite noticeable and comparable to the standard group in case of 500 mg/kg test drug. The observed antipyretic effect was found to be sustained and lasted up to at least 5 h after oral administration of the drug sample in case of both the 300 and 500 mg/kg samples which showed similar pattern of antipyretic efficacy. Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. Mediators like interleukin 1β, α, β and TNF-α increase the synthesis of prostaglandin E2 near pre-optic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature. Flavonoids are known to target prostaglandins which are involved in pyrexia and also inhibit the effect of enzymes that are responsible for the inflammatory process (Pan et al., 2010; Binny et al., 2010; Owoyele et al., 2008).

The evaluation of acute inflammation which was induced by the carageenan in the paw of rats demonstrated the significant percentage decrease in circumference and oedema resulting from administration of the methanol extract of test drug in doses of 300 and 500 mg/kg. The percentage inhibition of acute inflammation in the paw of rats was found to be lower in case of 500 mg/kg of test drug dosage when compared to the standard drug indomethacine after 4 h of treatment. The acute and sustained effect on the induced inflammation up to four hours depends upon the decreased production of pro-inflammatory cytokines and prostaglandin E_2 (PGE_2) in the tissue of the effected part of the body. The inflammation induced by phlogostic agent is related to the production of histamine, bradykinin and cyclooxygenase products while delayed phase is related to neutrophil infiltration, as well as to the continuing of the production of arachinoic acid metabolites. Prostaglandins and nitric oxide biosynthesis is involved in inflammation, and isoforms of inducible nitric oxide synthase (iNOS) and of cyclooxygenase (COX-2) are responsible for the production of a great amount of these mediators. It has been demonstrated that flavonoids are able to inhibit both enzymes, as well as other mediators of the inflammatory process such as reactive C protein or adhesion molecules

(Gonzalez et al., 2007).

The variety of flavonoids have been found to have antimicrobial, antiviral, anti-ulcerogenic, cytotoxic, anti-neoplastic, mutagenic, antioxidant, antihepatotoxic, ant-ihypertensive, hypolipidemic, antiplatelet and antipyretic anti-inflammatory activities. Flavonoids also have biochemical effects, which inhibit a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca (+2)-ATPase, lipoxygenase, cycloxygenase, etc. (Pan et al., 2010; Owoyele et al., 2008; Rathee et al., 2009).

The detailed scientific evaluation of the pharmacological properties of the methanolic extract of the test drug in terms of its analgesic, antipyretic and anti-inflammatory actions clearly brought forth its significant therapeutic efficacy which was found to be comparable to that of the standard drugs. The test drug was also found to be significantly effective and having sustained effect along with no noticeable acute toxicity during this pre-clinical study. These three properties which have been known to be inter-related and inter-dependent could be attributed to the presence of flavonoids in the methanolic abstract. Hence, the results obtained during this research could be taken as a benchmark, and detailed further studies could be undertaken in the domain of phyto-chemistry and chemical analysis to identify the actual responsible bio-chemical marker compounds in the test drug. The findings and outcome of this research study could also form the basis for future clinical trials involving this research drug to establish its safety and efficacy in human subjects in view of its significant and sustained therapeutic activities documented during this study using rodents.

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