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In-vivo evaluation of analgesic, anti-inflammatory and anti-pyretic activity of aqueous methanolic extract of *Jatropha gossypifolia*

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Many pathological conditions are associated with pain, fever and inflammation. Synthetic drugs available for the treatment of these ailments accompany many unwanted side effect; most prominent of which is the gastric ulcer. Present study was thus aimed at evaluating the analgesic, anti-pyretic and anti-inflammatory potential of aqueous methanolic extract of leaves of plant *Jatropha gossypifolia*. Anti-inflammatory activity was evaluated using carrageenan induced paw edema. Acetic acid induced writhing test and hot plate method was used to assess the analgesic activity. Anti-pyretic activity was ascertained using brewer's yeast induced pyrexia. The aqueous methanolic extract of the plant *J. gassypifolia* have demonstrated significant analgesic, anti-pyretic and anti-inflammatory activity at 200 mg/kg dose. The data was statistically analyzed by one way ANOVA followed by post hoc Dunnett's test for multiple comparisons. It has thus concluded that aqueous methanolic extract of leaves of plant *J. gassypifolia* possess analgesic, anti-inflammatory and anti-pyretic properties. These results strongly support the ethno-pharmacological use of this plant as anti-pyretic, analgesic and anti-inflammatory agent.

Key words: *Jatropha gossypifolia*, anti-inflammatory, analgesic, anti-pyretic.

INTRODUCTION

Plant derived chemicals have been used by men for centuries as a source of medicine (Semwal et al., 2010). A large in number of the population in Pakistan has been using these medicinal plants as their primary source of

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medicine health care system (Shinwari, 2010). The use of such medicinal plants as remedy against various ailments is known by the name of hikmat. Approximately 40,000 of such registered hakims are practicing as health care professional using plant extracts for curing various diseases (Saeed et al., 2011). Practicing as hakim, have no scientific bases therefore needs validation on scientific basis (Khan et al., 2014). Jatropha gossypifolia plant belongs to the family “Euphorbiaceae” is locally known as “Lal bherandha”. This plant family possesses about 170 different types of species (Kamal et al., 2011). It is extensively found in southern region of Pakistan. The plant has long been used as astringent, anti-cancer, analgesic, anti-inflammatory, diaphoretic and as anti-feedant agent. The leaf bath is used for sores, sprains and rashes. Also the decoction of the leaves is useful for stomach ache, venereal diseases and as a blood purifier (Vickers, 2001). Furthermore this plant was also been used in various skin related ailments and some blood related disorders. The leaf extract has been used as an anticoagulant for biochemical and hematological analysis (Oduola et al., 2004). Whole plant including seeds, flowers, fruit and leaves have medicinal properties. In the present study the aqueous methanolic extract of leaves of the plant *J. gassypifolia* had been used for its potential analgesic, anti-inflammatory and anti-pyretic activity in animal models.

MATERIALS AND METHODS

Chemicals

Indomethacin was purchased from Liometacin Chesi Pharmaceutical, Pakistan. Paracetamol (Provas inj) and Tramadol (Tonoflex inj) were purchased from SAMI Pharmaceuticals Pvt. Ltd. Dimethyl sulfoxide liquid (DMSO), acetic acid, and Brewer’s yeast were purchased from Lohari Laboratory Chemicals Pvt. Ltd. Lahore Pakistan; Carrageenan was purchased from Sigma lumda, USA. Sterile water for injection, normal saline (Medisol pharmaceuticals) were obtained from Baber Medicine Company, Kot lakhpatt, Lahore, Pakistan. DMSO was used as control vehicle in all the experiments and all the dilutions of the plant extract were prepared in DMSO.

Animals

The experiments were carried out in albino rats (aging between 40 and 50 days) of species *Rattus norvegicus* of either sex weighing 130 to 150 g. The rats were locally purchased from University of Health Sciences Lahore. The rats were maintained in standard laboratory conditions of 22 to 25°C with alternate light/dark 12/12 h periods. The rats were feed in pellet form and water *ad libitum*.

Plant materials

Leaves of the plant *J. gassypifolia* were collected locally in the month of July to August, 2013. Specimen of the subject plant was identified by Taxonomist in the Department of Botany Govt. College University Lahore and a specimen was also submitted there in the herbarium with voucher number 2235/Bot. The extract was prepared and standardized as described by Jabeen et al. (2009), briefly; the leaves approximately 700 g were sunshade dried for 14 days and then powdered. The powdered material was then dissolved in 70% methanol with gentle shaking thrice every day for 7 days. The extract was then filtered through filter paper and concentrated using rotary evaporator at low temperature (Jabeen et al., 2009).

Analgesic activity

Acetic acid induced writhing test

Albino rats were divided randomly into five groups comprising of 4 rats each. The rats were deprived of feed 6 h before experiment. Group I received control DMSO liquid 10 ml/kg, group II received indomethacin 10 mg/kg and the III, IV and V groups received *J. gassypifolia* leaves extract 50, 100 and 200 mg/kg body weight through intra-peritoneal route respectively. After 1 h of administration of DMSO, indomethacin and plant extract all the rats were injected with 1% acetic acid solution intra-peritoneal. The number of writhing was counted for 20 min after acetic acid injection as described by (Hajare et al., 2000).

Hot plate test

Experimental rats were acclimatized to laboratory conditions and were randomly divided into five groups consisting to 4 rats in each group. Animals were deprived of feed 1 h prior testing procedure. All the rats were pre-tested for measuring the latency time on hot plate which was maintained at 55±2°C. Animals showing latency time greater than 15 s were rejected from the experiment. Group I received control DMSO liquid 10 ml/kg, group II received tramadol 20 mg/kg and III, IV and V groups received plant extract 50, 100 and 200 mg/kg body weight respectively. Latency time of lifting the paw was then noted by placing each rat on the hot plate maintained at 55±2°C at 0, 20 and 60 minutes after treatment as described by Hajare et al. (2000).

The analgesia percentage was then calculated using the formula:

% Analgesia = (Test latency-Control latency) /cut off time-controlled latency) × 100

Anti-inflammatory activity

Carrageenan induced paw edema

The anti-Inflammatory activity of aqueous methanolic extract of leaves of the plant *J. gassypifolia* was determined using carrageenan as inflammatory mediator (Amdekar et al., 2012). Albino rats (aging between 40 and 50 days) of species *Rattus norvegicus* of either sex weighing 130 to 150 g were divided randomly into five groups consisting of 4 rats in each group. All rats were deprived of feed 1 h before experimentation. Group I rats were treated with DMSO liquid as negative control, group II rats were treated with standard drug indomethacin 10 mg/kg body weight, group III, IV and V were treated with *J. gassypifolia* extract 50, 100 and 200 mg/ kg body weight respectively. After 1 h of intra-peritoneal injection of all the groups, 1% carrageenan solution of approximately 0.5 ml was injected into the left hind paw of each rat. Paw volume of each rat was measured immediately at 0 h and after 3 h of carrageenan immersion injection using liquid immersion method (Fereidoni et al., 2000). The average paw edema in plant

Table 1. Effect of *Jatropha gossipifolia* aqueous methanolic extract 50, 100 and 200 mg/kg in acetic acid induced writhing test.

Animal	Average no. of writhing	Decrease in writhing	Percentage inhibition
Group I negative control (DMSO) 10 ml/kg	25+4.8	0	0
Group II positive control (Indomethacin10 mg/ kg)	13+1.25*	12	48
Group III JG-Cr treatment group (50 mg/kg)	21+4.5	4	16
Group IV JG-Cr treatment group (100 mg/kg)	19+3.4*	6	24
Group V JG-Cr treatment group (200 mg/kg)	17+1.4*	8	32

The data was analyzed by one way ANOVA followed by post hoc Dunnett's test for multiple comparisons. The data are reported as mean ± S.E.M for group of 4 animals. Asterisks indicated the statistically significant values as compared to control $p < 0.05$.

Table 2. Central analgesic effect of *J. gossipifolia* extract at 50, 100 and 200 mg/kg.

Treatment	Average latency time (s)			
	0 min	20 min	60 min	% Analgesia
DMSO 10 ml/kg	4.85±0.25	4.6± 0.81	4.6± 0.24	0
Tramadol 20 mg/kg	4.52± 0.43	6.4± 0.26*	8.9± 0.28*	93
JG-Cr 50 mg/kg	4.52± 0.62	4.9± 0.26	5.1± 0.43	11
JG-Cr 100 mg/kg	4.47± 0.40	5.2± 0.37	5.5± 0.33*	29
JG-Cr 200 mg/kg	4.45± 0.53	5.2± 0.25	6.3± 0.4*	58

The data was analyzed by one way ANOVA followed by post hoc Dunnett's test for multiple comparisons. The data are reported as mean ± S.E.M for group of 4 animals. Asterisks indicated the statistically significant values as compared to control *$p < 0.05$.

extract treated groups and group that was treated with the standard was compared with that of negative control group (Gupta et al., 2003).

Inhibition of the inflammation was then measured using following formula:

$$Percentage\ inhibition = \frac{(\mathrm{Vt} - \mathrm{Vo})\mathrm{control} - (\mathrm{Vt} - \mathrm{Vo})\ \mathrm{treated}}{(\mathrm{Vt} - \mathrm{Vo})\mathrm{control}} \times 100$$

Where Vt is paw volume at time t and Vo is volume at zero time.

Anti-pyretic activity

Brewer's yeast induced pyrexia

Anti-pyretic activity of plant extract was determined using brewer's yeast suspension induced pyrexia in animal models. The normal rectal temperature of each rat was measured with the help of clinical thermometer. All the rats were then injected subcutaneously (at the nape of neck) with 20% brewer's yeast suspension at a dose of 20 ml/kg body weight. After 24 h of injecting yeast suspension rectal temperature of all the rats was again measured using clinical thermometer.

All the rats were randomly placed into five groups consisting of 4 rats each. Group I received negative control DMSO liquid 10 ml/kg, group II received standard drug paracetamol 150 mg/kg and the III, IV and V groups were given plant extract 50, 100 and 200 mg/kg body weight through intra-peritoneal route respectively. Rectal temperature was then measured immediately at 0hr and after 1, 2, 3 and 4 h after drug treatment (Bajpai et al., 2014).

Statistical analysis

The data was statistically analyzed using one way ANOVA followed by post hoc Dunnett's test for multiple comparisons.

Animal experiment ethics

All animal handling procedures received approval from the Animal Management Ordinance of the Pakistan; and all the animal experiment standards approved by the Animal ethics committee of University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

RESULTS

Analgesic effect of *J. gassypifolia* extract

The study showed that the plant extracts of *J. gassypifolia* demonstrated significant peripheral as well as central analgesic activity, at a dose level of 200 mg/kg of body weight (Khan, 1992). The analgesic activity of the extract for peripheral analgesic activity was found to be 32% at a dose level of 200 mg/kg as compared to 48% for indomethacin taken as standard treatment (Table 1).

The central analgesic activity was evaluated and the plant extract of *J. gassypifolia* showed dose dependent increase in latency time and the analgesic effect was found to be 58% at 200 mg/kg dose as compared to 93% for reference drug tramadol 20 mg/kg (Table 2). The

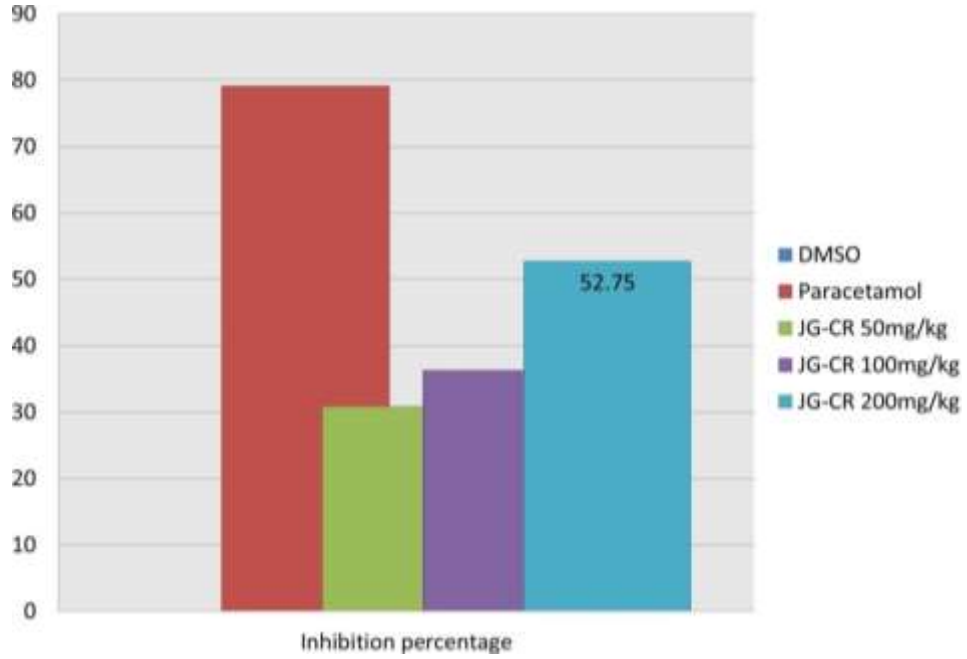

Figure 1. Percentage inhibition of inflammation after three hours of treatment; effect of intraperitoneal administration of *Jatropha gossypipifolia* aqueous methanolic extract at 50, 100 and 200 mg/kg doses in carrageenan induced paw edema test. The data was analyzed by one way ANOVA followed by post hoc Dunnett's test for multiple comparison; the data are reported as mean ± S.E.M for group of 4 animals. Asterisks indicated the statistically significant values as compared to control p <0.05.

Table 3. Effect of intraperitoneal administration of *J. gossypipifolia* aqueous methanolic extract at 50, 100 and 200 mg/kg doses in carrageenan induced paw edema test.

Animal	Average paw volume cm^3 at 0 h	Average paw volume cm^3 at 3 h	Average increase in paw volume cm^3 after 3 h (vt-vo)	Decrease in oedema as compared to negative CTRL
Group I negative control (DMSO) 10 ml/kg	0.419 ± 0.04	1.324 ± 0.04	0.91	0
Group II positive control Indomethacin 10 mg/kg	0.379 ± 0.045	0.573 ± 0.05	0.19	0.72
Group III JG-Cr 50 mg/Kg	0.426 ± 0.03	1.059 ± 0.03	0.63	0.28
Group IV JG-Cr 100 mg/Kg	0.441 ± 0.027	1.017 ± 0.03	0.58	0.33
Group V JG-Cr 200 mg/Kg	0.424 ± 0.03	0.852 ± 0.03*	0.43	0.48

The data was analyzed by one way ANOVA followed by post hoc Dunnett's test for multiple comparison the data are reported as mean ± S.E.M for group of 4 animals. Asterisks indicated the statistically significant values as compared to control p <0.05.

percentage analgesia was calculated using the following formula:

Percentage Analgesia = (test latency - control latency/cut off time- control latency × 100)

Anti-inflammatory effect of plant extract

In carrageenan induced paw edema the plant extract of *J. gassypifolia* showed dose dependent anti-inflammatory activity. The maximum anti-inflammatory activity of the plant extract *J. gassypifolia* was found at a dose level of 200 mg/kg of about 53% (Figure 1) as compared with the reference drug indomethacin 10 mg/kg where the anti-inflammatory activity was 79% (Table 3).

Anti-pyretic effect of the plant extract

The plant extract at 200 mg/kg showed significant anti-pyretic activity in brewer's yeast induced pyrexia in

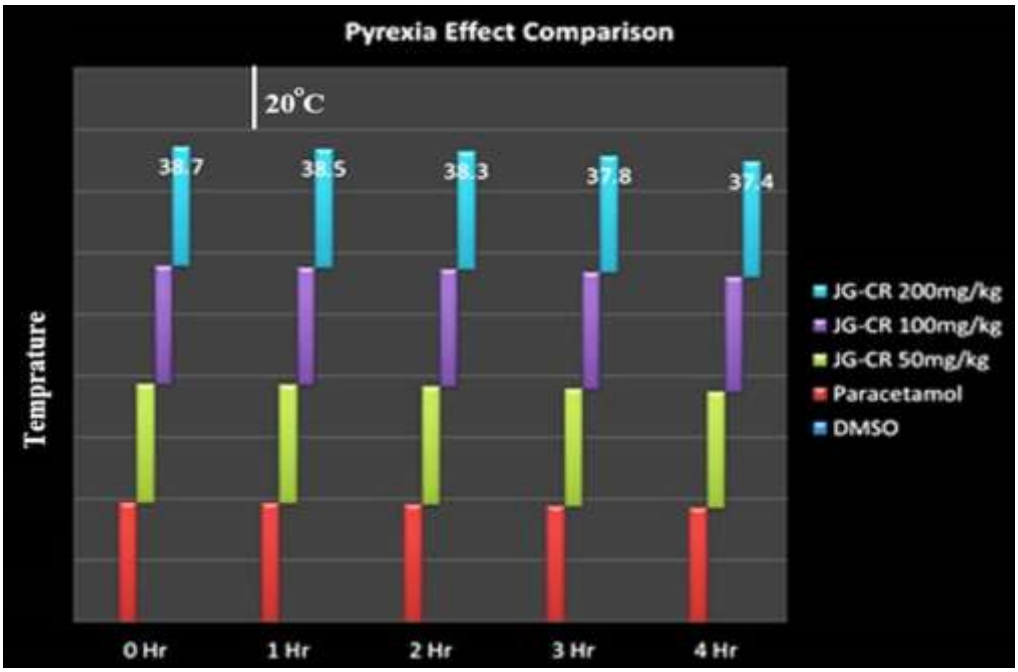

Figure 2. Effect of intraperitoneal administration *J. gassypifolia* aqueous methanolic extract 50, 100 and 200 mg/kg in yeast induced pyrexia in rats. The data was analyzed by one way ANOVA followed by post hoc Dunnett's test for multiple comparison; the data are reported as mean ± S.E.M for group of 4 animals. Asterisks indicated the statistically significant values as compared to control *p<0.05.

Table 4. Effect of intraperitoneal administration *J. gassypifolia* aqueous methanolic extract 50, 100 and 200 mg/kg in yeast induced pyrexia in rats.

Group treatment	Initial rectal temperature (°C)	Rectal temperature after treatment (°C)					
		0 hour	1 hour	2 hour	3 hour	4 hour	% inhibition
DMSO liquid 10 ml/kg	36.7±0.11	38.6 ±0.34	38.5 ±0.21	38.4 ±.11	38.4 ±0.20	38.2 ±0.20	21
Paracetamol 150 mg/kg	36.7 ± 0.08	38.8 ± 0.04	38.5 ± 0.01	38.1 ± 0.02	37.7 ± 0.28	37.0± 0.04*	85
JG-Cr 50 mg/kg	36.8 ± 0.02	38.5 ± 0.43	38.5 ± 0.22	38.3 ± 0.22	38.1 ± 0.11	37.8 ±0.12	41
JG-Cr 100 mg/kg	36.6 ± 0.14	38.5 ± 0.11	38.3 ± 0.02	38.1 ± 0.25	37.9 ±0.11	37.4 ± 0.41*	58
JG-Cr 200 mg/kg	36.7 ± 0.12	38.7 ± 0.20	38.5± 0.31	38.3 ± 0.21	37.8 ± 0.10	37.4± 0.23*	65

The data was analyzed by one way ANOVA followed by post hoc Dunnett's test for multiple comparison The data are reported as mean ± S.E.M for group of 4 animals. Asterisks indicated the statistically significant values as compared to control *p <0.05

animal model. The rectal temperature of rats was recorded at 0, 1, 2, 3 and 4 h after the drug treatment (Figure 2). The results thus depicted that the aqueous methanolic extract of *J. gassypifolia* leaves illustrated significant anti-pyretic activity (65%) at dose of 200 mg/kg comparable to the reference drug paracetamol which was 85% (Figure 3) at a dose of 150 mg/kg (Table 4).

DISCUSSION

Results of the present study revealed that leaves of the plant *J. gassypifolia* possess significant analgesic, anti-pyretic as well as anti-inflammatory activity. Carrageenan induced edema is known to be the acute inflammatory model; sensitive to the cyclooxygenase inhibitors and is used to find the effects of non-steroidal anti-inflammatory agents which inhibit cyclooxygenase involved in prostaglandin synthesis. Although both pathways, that is, cyclooxygenase and lipoxygenase are involved in mediating inflammatory process but cyclooxygenase inhibitors are considered more effective than lipoxygenase inhibitors (Ndebia et al., 2007).

Carrageenan induced paw edema model is well

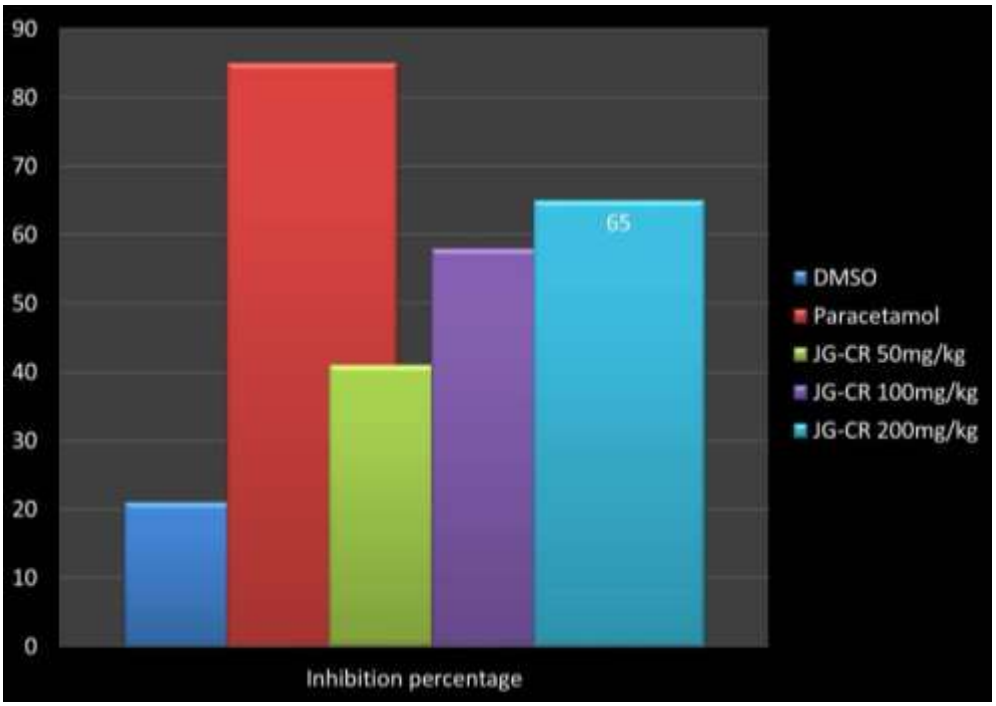

Figure 3. Percentage inhibition of pyrexia after 4 h of the treatment, effect of percentage inhibition of pyrexia with intraperitoneal administration *J. gassypifolia* aqueous methanolic extract 50, 100 and 200 mg/kg in yeast induced pyrexia in rats. The data was analyzed by one way ANOVA followed by post hoc Dunnett's test for multiple comparison; the data are reported as mean ± S.E.M for group of 4 animals. Asterisks indicated the statistically significant values as compared to control *p <0.05.

established technique for the estimation of anti-inflammatory activity for plant derived natural substances as well as for synthetic drugs. The edema formation is a two phase process; the initial phase of edema involves the action of inflammatory mediators on the vascular permeability, including histamine, serotonin and bradykinin (Toni et al., 2015). The second or the later phase involves the pain mediators like prostaglandins (Chao et al., 2009). Pre-treatment of experimental animals with respective concentration of plant extract reflects that the plant extract was effective in attenuating the early phase of inflammation which involves the release of inflammatory mediators like histamine, serotonin and bradykinin (Muhammad et al., 2012). When the edema was induced by injecting 1% carrageenan solution into the left hind paw of all the experimental rats (Gupta et al., 2003), the aqueous methanolic extract of *J. gassypifolia* showed significant anti-inflammatory activity in both phases of inflammation at a concentration of 200 mg/kg body weight (Figure 1).

The analgesic activities of plant derived natural substances are usually evaluated using acetic acid induced writhing test model (Kumar et al., 2015). The pain induction is caused by the release of certain endogenous substances e.g. bradykinin and also from the production of pain mediators like arachidonic acid via cyclo-oxygenase pathway and prostaglandin synthesis (Verma et al., 2015). Abdominal writhing usually involves certain pain receptors which are located in the peritoneum (Mbiantcha et al., 2011). When peripheral analgesic activity was determined by acetic acid induced writhing test as described by Hugar et al. (2010), the results demonstrated that the plant extract of *J. gassypifolia* significantly reduces the abdominal writhing in rats at a dose of 200 mg/kg indicating its activity on local peritoneal pain receptors (Table 2).

The hot plate method is a common technique usually used in measuring the central analgesic effects (Singh et al., 2015). The results showed that the plant extract of *J. gassypifolia* at a concentration of 200 mg/kg body weight significantly increased the latency time of lifting the paw which is comparable to tramadol 20 mg/kg used as control. The increase in latency time of lifting the paw as compared with the negative control reflected the decrease in pain threshold which might be due to the inhibition or suppression of thermo receptors in the brain region. The plant extract *J. gassypifolia* at 200 mg/kg dose showed 58% increase in latency time of lifting the paw as compared to the standard drug tramadol which reflected 93% increase in latency time at a dose level of 20 mg/kg (Table 2).

Administration of 20% yeast suspension below the

nape of the neck to the experimental animals induces pyrexia possibly by increasing the synthesis of prostaglandins (Eldahshan and Abdeldaim, 2015). The induction of fever by this method is called pathogenic fever. Induction of pyrexia or fever involves liberation of several mediators and hence antipyretic effect can only be achieved by inhibiting or blocking the release of these mediators. In present study, the plant extract of *J. gassypifolia* has shown its significant anti-pyretic activity (65%) at dose level of 200 mg/kg indicating the presence of chemicals in plant extract that are involved in the inhibition of prostaglandins synthesis confirming the plants potential as antipyretic agent(Figure3and Table 4).

In conclusion the extract of the plant *J. gassypifolia* was proved to be a natural and effective remedy for the treatment of pain, inflammation and fever. The study has thus justified the folk use of the *J. gassypifolia* plant extract as a remedy for clinical signs, including pain, inflammation and fever on scientific basis and may require further isolation of active ingredient out of extract for the advancement in pharmaceutical sciences.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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