Mashood Ahmed Sangreskopp. *et al*. / *International Journal of Phytopharmacology*, 5(1), 2014, 38-45.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

EVALUATION OF ANTI-PYRETIC AND ANTIMICROBIAL POTENTIALS OF *SIDA SPINOSA* LINN. ETHANOLIC ROOT EXTRACT IN RATS WITH POSSIBLE MECHANISM

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ABSTRACT

Study was conducted to understand the Antipyretic and antimicrobial potential and mechanism of action of *Sida spinosa* Linn. Ethanolic root extract in experimental animals. Roots were extracted with ethanol by successive extraction in soxhlet apparatus. The doses of the extract selected were 200 and 400 mg/kg b.w. Antipyretic potential was evaluated in Brewer's yeast and 2,4-dinitrophenol induced pyrexia in rats along with Antimicrobial activity by agar well diffusion technique, MIC and MBC. Ethanolic extract (400mg/kg b.w) demonstrated highly significant ($P<0.01$) antipyretic activity when challenged in both yeast and 2,4-dinitrophenol induced pyrexia. Maximum attenuation in yeast and 2,4-dinitrophenol induced pyrexia were 74.12 % and 65.73% at 3h. Antimicrobial activity against *S. aureus, B. subtilis, E. coli* and *S. aeruginosa*, was carried out by disc diffusion and micro dilution methods. All microbes were sensitive and activity was concentration dependent. *Sida spinosa* root extract shown potent antipyretic activity in yeast and 2,4-dinitrophenol induced pyrexia, supported by its antimicrobial potential which is comparable with standard aspirin & has therapeutic potential.

Key words: *Sida spinosa*, Antipyretic & Antimicrobial activity, Minimum inhibitory concentration (MIC), Minimum bacterial concentration (MBC), Aspirin, Tetracycline.

INTRODUCTION

Fever is a complex physiologic response triggered by infections or aseptic stimuli. Elevation in body temperature occurs when the concentration of prostaglandin E2 (PGE2) increases within parts of the brain (Flower RJ, Vane JR, 2004). Pyrexia is caused in response to infection, tissue damage, inflammation and other diseased conditions (Chattopadhyay D *et al*., 2005). All these conditions enhance formation of cytokines such as IL-1b, IL-6 (Interleukin), interferon's, and TNF-α. The cytokines increase synthesis of PGE2 in circumventricular organs in and adjacent to the preoptic hypothalamic area; PGE2, in turn, increases cyclic AMP and triggers the hypothalamus to elevate body temperature by promoting an increase in heat generation and a decrease in heat loss. Aspirin suppress this response by inhibiting PGE2 synthesis (Goodman & Gillman, 2003). Yeasts are eukaryotic microorganisms. The yeast cells when injected sprout a hyphal outgrowth, which locally penetrates the mucosal membrane, causing irritation and shedding of the tissues (Anonymous 1). The earlier study have demonstrated potent antimicrobial activity of ethanolic extract of *Sida spinosa* Linn. Leaf and whole plant (Navaneethakrishnan S *et al*., 2011; Selvadurai S *et al*., 2011). Ethno botanical survey conducted by C.P. Khare reveals that roots of *Sida spinosa* are used as nervine tonic and diaphoretic, in debility and fevers (Khare CP, 2007).

The investigation of medicinal properties of various plants attracted an increasing interest since last couple of decades because of their potent pharma

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cological activities, convenience to users, economic viability and low toxicity (Prashant KR *et al*., 2008). This regained interest to plant-derived medicines is basically due to the multidrug resistance of many antibiotics as well as current widespread perception that green medicine is safe and dependable than the expensive synthetic drugs most of which have adverse effects (Jigna P & Sumitra C, 2006).

A number of medicinal plants have been screened for antimicrobial activity in recent years and efforts have been done to identify their active constituents (Premanth R *et al*., 2011; Tijjani MB *et al*., 2009). Many infectious diseases are known to be treated with herbal remedies. Antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Bhagyalakshmi B *et al*., 2009).

Recent data suggests that 80% drug molecules are natural products or natural compound inspired (Kamlesh KB, Vikrantsinh MG, 2008).

MATERIAL AND METHODS

Drugs and Chemicals

Aspirin, Brewer's Yeast, Carboxymethyl cellulose, Tetracycline and 2,4-Dinitrophenol were purchased from Sigma Aldrich, Himedia and S.D Fine chemicals. All chemicals used were of analytical grades.

Plant material and preparation of extract

Roots of *Sida spinosa* Linn. were collected from surrounding areas of Dharwad and authenticated by Dr. Hebbar, Professor, Government PU College, Dharwad. The powdered roots were extracted in a Soxhlet apparatus with ethanol by maintaining temperature at 50^0C for 12 h. The resultant extract was filtered, then concentrated to dryness in a rotary evaporator under reduced pressure at 40^0C. The dried mass was stored in a desiccators until use (Prince PSM *et al*., 2004; Rahmana MT *et al*., 2002).

Preliminary Phytochemical investigation

The preliminary phytochemical investigation of ethanolic root extract was carried in accordance with the standard methods described in practical pharmacognosy by K.R. Khandelwal (2004).

Animals

Albino Wister rats of either sex weighing 150–200 g were used for the study. Animals were maintained under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 5%) and 12-h light-dark cycles, fed with commercial stock diet and water, *ad labium*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SET's College of Pharmacy, Dharwad, India (REG.No.112/1999/CPCSEA) according to prescribed guidelines of CPCSEA, Government of India.

PHARMACOLOGICAL EVALUATION

Acute toxicity study

The acute oral toxicity study was carried out as per the guidelines set by OECD 423. Animals (n=3) were fasted overnight prior to dosing. The starting dose used was 5mg/kg b.w. Ttest substance was administered in a single dose by gavage using intubation canula. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. The suspension of ethanolic extract was prepared freshly by using tragacanth (2%) in distilled water. The extract was administered at a constant volume of 10 ml/kg for each animal (OCED, 2002).

ANTIPYRETIC ACTIVITY

Effect on yeast-induced pyrexia

Group 1: Distilled Water (Normal Control)
Group 2: Brewer's yeast suspension (10 ml/kg, s.c.) (Positive Control)
Group 3: Brewer's yeast suspension (10 ml/kg, s.c.) + Extract (200 mg/kg p.o.)
Group 4: Brewer's yeast suspension (10 ml/kg, s.c.) + Extract (400 mg/kg p.o.)
Group 5: Brewer's yeast suspension (10 ml/kg, s.c.) + Aspirin (100 mg/kg p.o.)

Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. Hyperthermia was induced in rats by injecting 20% w/v suspension of Brewer's yeast(10ml/kg, s.c.) in 0.9% saline into the animals dorsum region, by using digital clinical thermometer (Hartmann, Germany) rectal temperature were recorded initially and at 17h. Animals with 0.7^0C or more elevation in body temperature were used for study. The animals received the test compound, standard drug and vehicle orally after 17h of yeast injection. Rectal temperature was measured at 1 h intervals up to 5 h after administration of drug or plant extracts (Paschapur MS *et al*., 2009).

2, 4-Dinitrophenol (DNP) induced hyperthermia

Overnight fasted rats were used for the experiment. They were randomized into groups of 6 rats each. DNP (10 mg/kg, i.p.) was administered to the rats after obtaining the basal rectal temperatures. Hyperthermia developed within 30 min of DNP administration. Different doses of extract (200 and 400 mg/kg, o.p.), aspirin (100 mg/kg) and distilled water (10 ml/kg, orally) were administered respectively to the treatment and control groups of animals. Rectal temperature was measured at 1 h intervals up to 5 h after

administration of drug or plant extracts (Mbagwu HO *et al*., 2007).

ANTIMICROBIAL ACTIVITY

Disc diffusion method

The antimicrobial activity was assayed against 4 human pathogenic microorganisms using Brain Heart Infusion agar along with some modifications in method described in Clinical microbiology procedures handbook (Isenberg HD, 1992).

Gram-positive bacteria: *Staphylococcus aureus* ATCC 12598, *Bacillus subtilis* ATCC 6633 Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas* ATCC 25619 (Isenberg HD, 1992)

MICRO-DILUTION METHODS

Minimal Inhibitory Concentration (MIC) procedure

Minimum inhibitory concentration was measured by determining the lowest amount of extract or standard antibiotic needed to inhibit the visible growth (turbidity) of test microorganisms after 24 hours incubation period at 37^0C. This was done using 9 dilutions of each drug in Brain Heart Infusion (BHI) containing different concentrations of extracts, tetracycline Schwalve *et al*., 2007).

Minimum Bacterial Concentration (MBC)

To avoid the possibility of misinterpretation due to the turbidity of insoluble compounds if any, the MBC was determined by sub-culturing the above MIC serial dilutions after 24h in nutrient agar plates using 0.01ml loop and incubated at 370C for 24h. MBC was regarded as the lowest concentration that prevented the growth of bacterial colony on solid media (Hammond SM, Lambert PA, 1978).

Statistical analysis

All data was expressed as mean ± S.E.M. of 6 rats per group. Statistical analysis was performed using Graph pad prism. Parametric one way analysis of variance (ANOVA) followed by Turkey's post test. The minimal level of significance was identified at $P < 0.05$.

RESULTS

Table 1. Effect of SSE on yeast induced pyrexia

Group & Dose	BBT ^{0}C	Rectal Temperature (^{0}C) After 18h of Yeast Injection (Mean ± S.E.M)					
		0 h	1 h	2 h	3 h	4 h	5 h
Normal Control	36.98	37.14	37.22	37.37	37.57	37.75	37.75
Positive Control	37.25	38.31±0.33	38.42±0.24	38.59±0.16	38.68±0.18	38.76±0.27	38.85±0.23
SSE (200 mg/kg)	37.34	38.43±0.11	38.37±0.27 (11.96%)	38.32±0.13 (26.86%)	38.03±0.17* (51.74%)	38.15±0.16 (46.35%)	38.29±0.16 (40.62%)
SSE (400 mg/kg)	37.45	38.27±0.10	38.19±0.11 (36.75%)	38.08±0.06* (52.98%)	37.82±0.04** (74.12%)	37.92±0.10* (68.21%)	38.1±0.06* (59.37%)
Aspirin (100 mg/kg)	37.41	38.21±0.09	37.93±0.13 (54.70%)	37.79±0.13*** (71.64%)	37.58±0.11*** (87.41%)	37.55±0.17*** (90.06%)	37.51±0.19*** (93.12%)

Each value represents Mean ± S.E.M., for n=5. Values in parentheses indicate percentage reduction in rectal temperature and $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ compared to control values.

Table 2. Effect of SSE on 2,4-Dinitrophenol (DNP) induced hyperthermia

Group & Dose	BBT ^{0}C	Rectal Temperature (^{0}C) After 18h of Yeast Injection (Mean ± S.E.M)					
		0 h	1 h	2 h	3 h	4 h	5 h
Normal Control	36.98	37.14	37.22	37.37	37.57	37.75	37.75
Positive Control	37.25	38.31±0.33	38.42±0.24	38.59±0.16	38.68±0.18	38.76±0.27	38.85±0.23
SSE (200mg/kg)	36.93	38.31±0.12	38.00±0.06 (8.54%)	37.96±0.05 (23.13%)	37.75±0.16* (42.65%)	37.93±0.11 (33.77%)	38.08±0.13 (28.12%)
SSE (400mg/kg)	37.37	38.29±0.08	38.18±0.10 (29.91%)	38.13±0.07 (43.28%)	37.86±0.09** (65.73%)	38.02±0.07* (56.95%)	38.17±0.04 (49.37%)
Aspirin (20mg/kg)	37.41	38.21±0.09	37.93±0.13 (54.70%)	37.79±0.13*** (71.64%)	37.58±0.11*** (87.41%)	37.55±0.17*** (90.06%)	37.51±0.19*** (93.12%)

Each value represents Mean ± S.E.M., for n=5. Values in parentheses indicate percentage reduction in rectal temperature and $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ compared to control values.

Table 3. Agar well diffusion technique

Microorganism	Zone of inhibition (mm) *Sida spinosa* ethanolic extract				
	75 µl	50 µl	25 µl	Chloramphenicol (Standard)	Negative control
S. aureus	10mm	8mm	R	21mm	0.0
B. subtilis	16mm	13mm	9mm	25mm	0.0
Escherichia coli	15mm	13mm	R	14mm	0.0
Pseudomonas	19mm	15mm	R	40mm	0.0

R: Resistant Negative control: Brain Heart Infusion agar.

Table 4. MIC and MBC of *Sida spinosa* ethanolic extract and tetracycline (µg/ml)

Extract	*S. aureus*		*B. subtilis*		*Escherichia coli*		*Pseudomonas*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ethanolic Extract	0.4	1.4	0.2	1.0	0.2	1.2	0.8	3.2
Tetracycline	0.01	0.03	0.04	0.06	0.012	0.09	0.045	0.55

Fig 1. Effect of *Sida spinosa* Linn. Ethanolic root extract on yeast induced pyrexia in rats

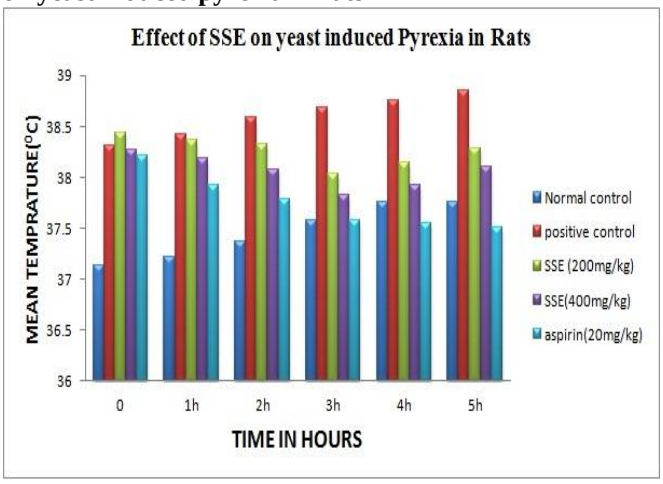


Fig 2. Percentage reduction of rectal temperature by SSE on yeast induced pyrexia in rats.

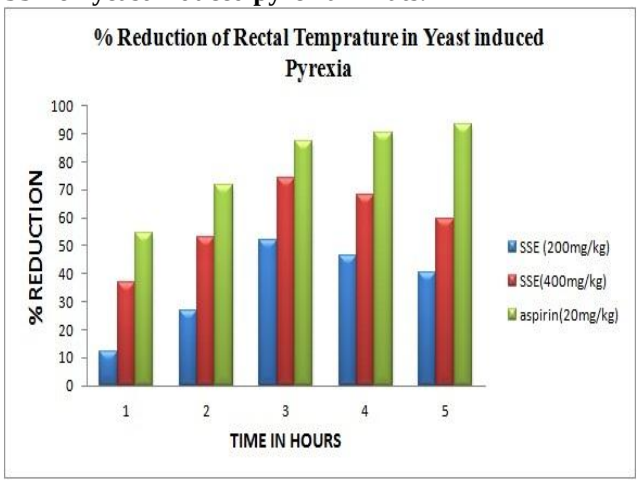


Fig 3. Effect of SSE on 2,4-Dinitrophenol induced Hyperthermia in Rats

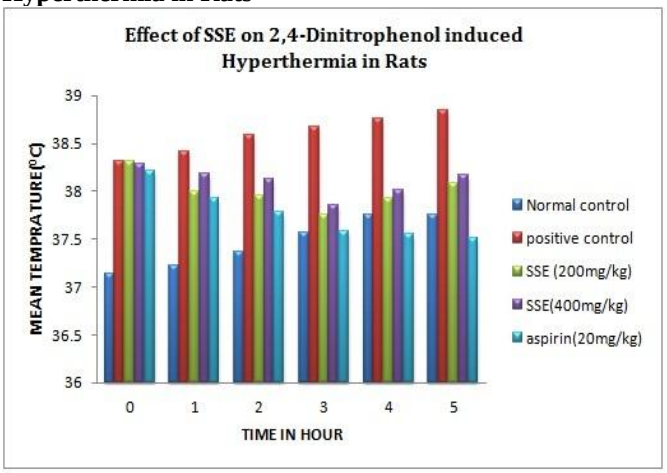


Fig 4. percentage reduction in rectal temperature by SSE in 2,4-Dinitrophenol induced Hyperthermia

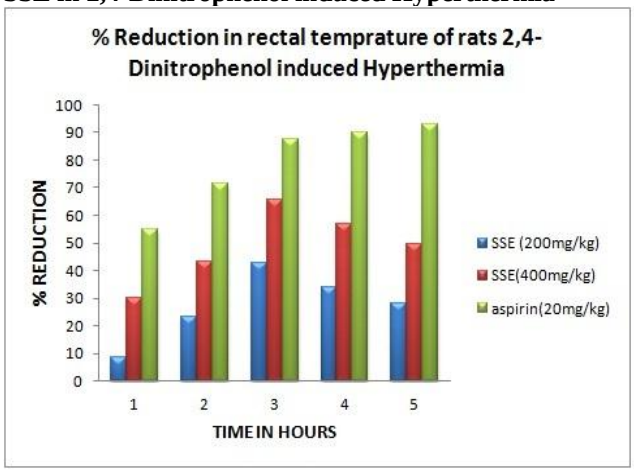


Fig 5. Zone of inhibition SSE against *Pseudomonas aeruginosa, Bacillus Subtilis, Staphylococcus Aureus and Escherichia Coli.*

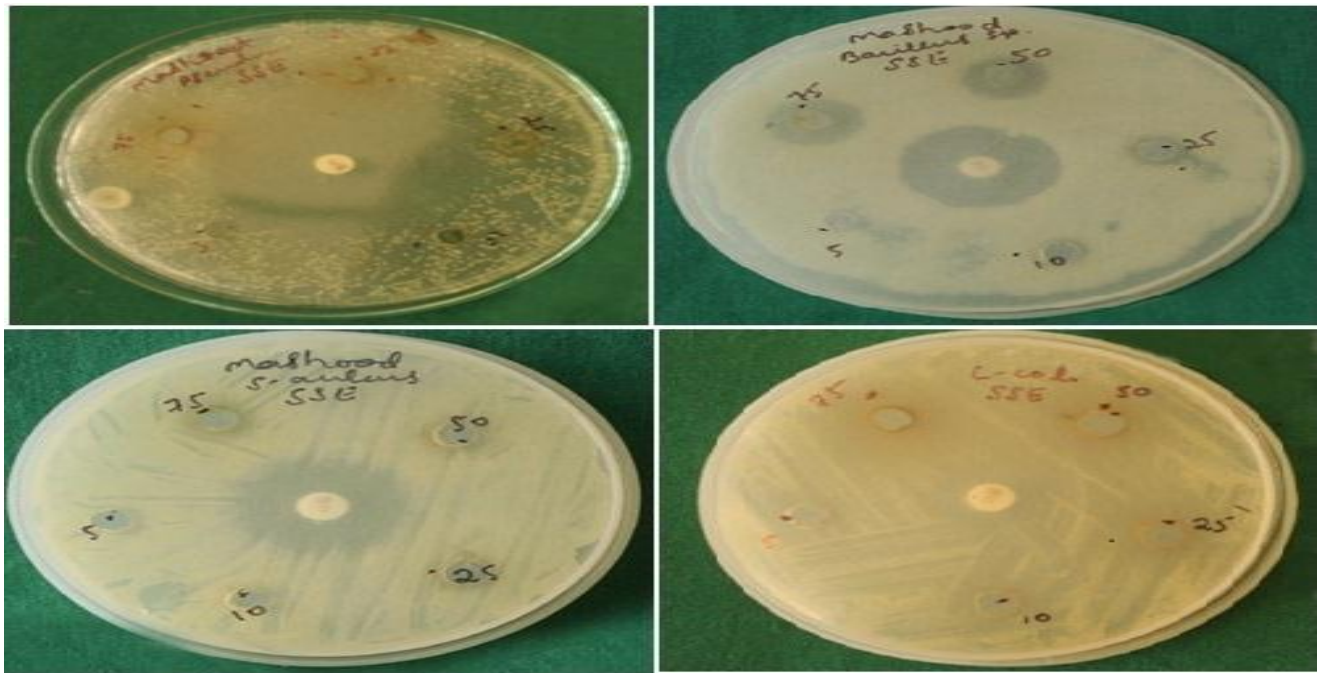
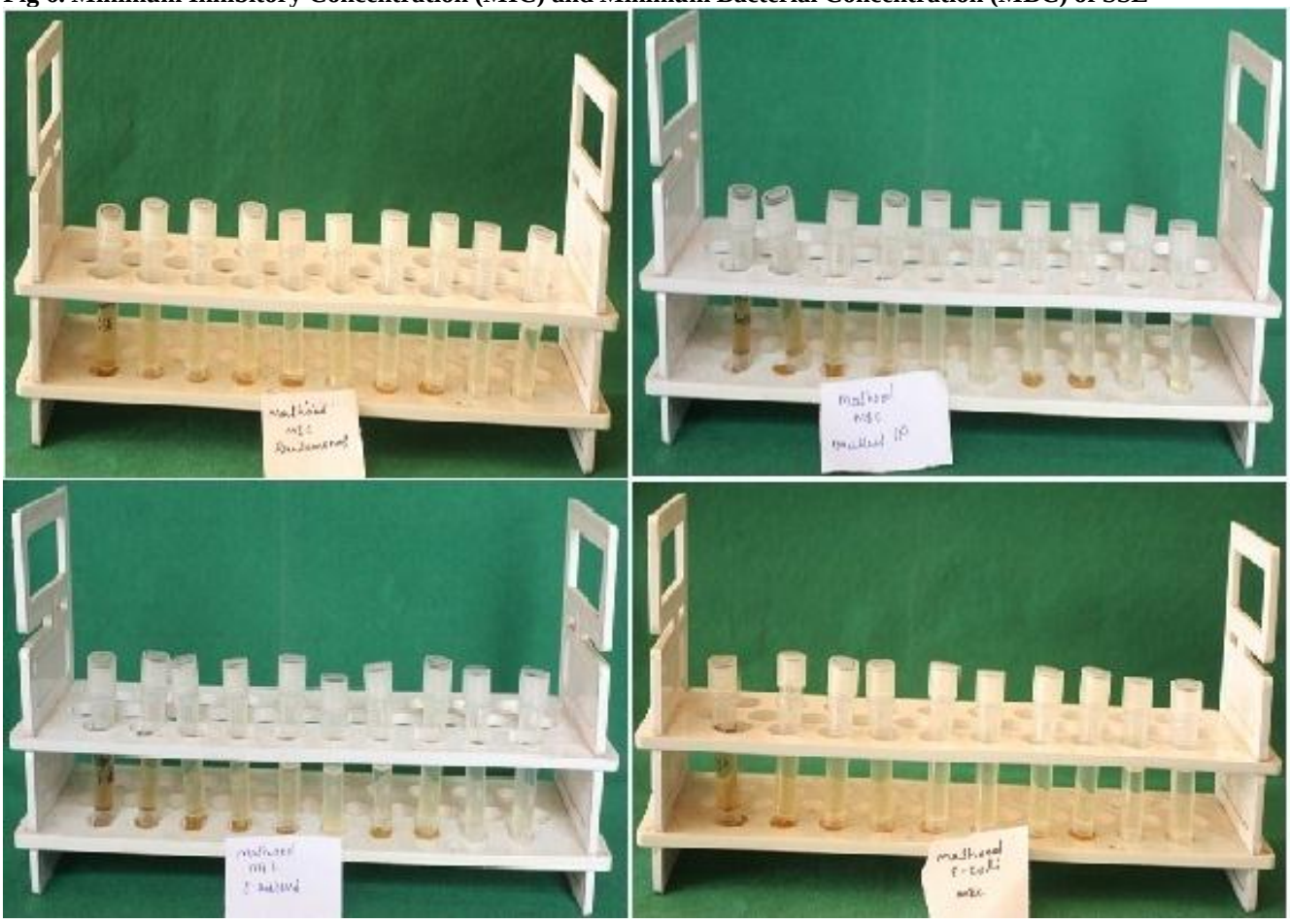


Fig 6. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of SSE



Phytochemical investigation

The phytochemical investigation of *Sida spinosa* Ethanolic extract showed the presence of phenolics, flavonoids and tannins.

PHARMACOLOGICAL EVALUATION

Acute oral toxicity studies

Acute toxicity study revealed that animals showed good tolerance (up to 4000 mg/kg b.w) to single dose of ethanolic extract. Extract produced no noticeable effect on general behavior or appearance of the animals and all rats survived during and after the test period. Therefore, two non-lethal, $1/20^{th}$ and $1/10^{th}$ of maximum tolerated doses (200 and 400 mg/kg b.w) of extract was selected for the above study.

ANTIPYRETIC ACTIVITY

Effect on yeast-induced pyrexia

Sida spinosa root Extract at higher dose (400 mg/kg) has significantly ($P<0.01$) attenuated Brewer's yeast induced hyperthermia (Table 1). The maximum percentage reduction in body temperature by extract was 74.12 % after 3 hours which is comparable with that of aspirin (87.41%) (Figure 1 & 2).

2,4-Dinitrophenol (DNP) induced hyperthermia

The antipyretic effect of *Sida spinosa* ethanolic root extract in DNP induced hyperthermia is shown in Table 2. Ethanolic extract at higher dose (400 mg/kg) showed significant ($P<0.01$) decrease (65.73% after 3h) in elevated body temperature when compared with the control group (Fig 3 & 4).

ANTIMICROBIAL ACTIVITY

Agar Well Diffusion Technique

Results of Disc diffusion method are shown in Table 3. The antimicrobial activity of extract was tested using three different concentrations of extract. All pathogens were sensitive, *Bacillus subtilis* being the most sensitive among all pathogens. The antimicrobial activity was concentration dependent. The *Sida spinosa* ethanolic extracts have shown zone of inhibition of 15 mm at 50 µl against *Pseudomonas*; 13 mm at 50 µl against *E. coli;* 9 mm at 25 µl against *Bacillus Sp* and 8 mm at 50 µl against *S. Aureus* (Figure 5).

MICRO DILUTION METHODS

Minimum inhibitory concentration (MIC) & MBC

The MIC and MBC values of tetracycline, ethanolic extract and negative control group are depicted in Table 4. MIC values of *Sida spinosa* ethanolic extract for *E. Coli* and *B. Subtilis* was observed at 0.2 µg/ml. MIC for *S. Aureus* was observed at 0.4 µg/ml, MIC for *Pseudomonas* was observed at 0.8 µg/ml (Fig 6). MBC values of *Sida spinosa* ethanolic extract for *E. Coli* and *B. Subtilis* was observed at 1.2 µg/ml. MBC for *S. Aureus* was observed at 1.4 µg/ml, MBC for *Pseudomonas* was observed at 3.2 µg/ml.

DISCUSSION AND CONCLUSION

Fever may be a result of infection, inflammation or other disease states. Yeast is eukaryotic microorganism; yeast-induced pyrexia is called pathogenic fever. Its etiology includes production of prostaglandins. Our present study exhibits dose-dependent significant suppression of yeast-induced pyrexia by SSE and indicates that it is the antipyretic effect. The antipyretic activity may be related to the inhibition of inflammatory mediator formation. Yeast injection causes irritation and shedding of the tissues, leading to the formation of inflammatory mediators, thereby causing hyperthermia. Phytochemical efficacy in attenuating hyperthermia may be due to inhibition of release of inflammatory mediators and antimicrobial potential.

Our results also exhibited that SSE significantly suppressed the growth of human pathogenic (*S. aureus, B. subtilis, E. coli, P. aeroginosa*) microorganisms in disc diffusion and micro dilution assays. This was supported by antimicrobial potential of SSE extract against four human pathogenic microorganisms and earlier studies have demonstrated potent antimicrobial activity of ethanolic extract of *Sida spinosa* Linn. Leaf and whole plant.

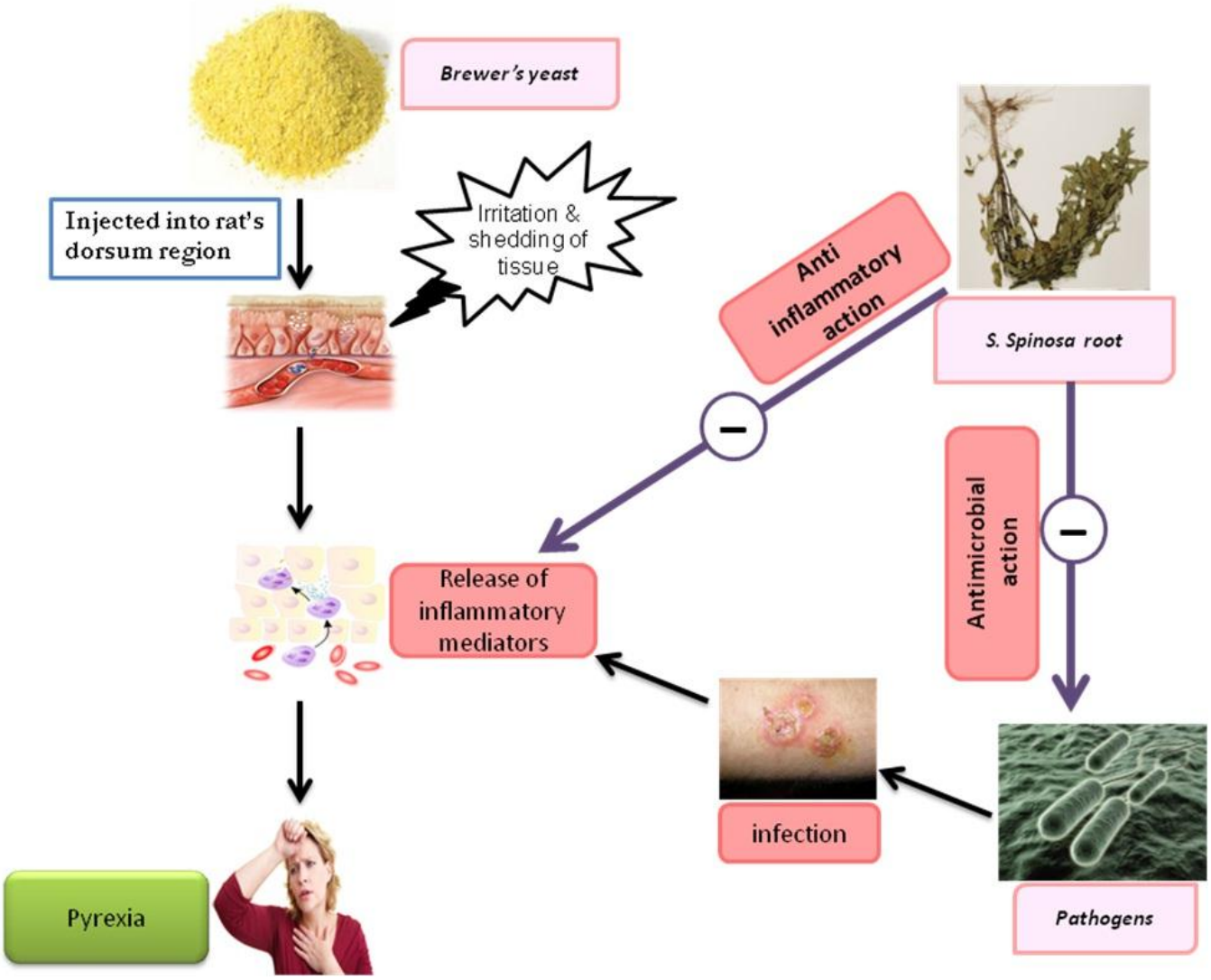
Based on these above results, we concluded that oral administration of SSE results in an anti- anti-pyretic effect by the following two mechanisms. First by inhibiting the release of inflammatory mediators and also by inhibiting the growth of microorganism. Phenolics, flavonoids and tannins are known to be biologically active because they protect the plant against infection.

The indiscriminate use of commercial antimicrobial drugs has caused multiple drug resistance in human pathogenic microorganisms (Aliero A *et al*., 2008). In addition to this problem hypersensitivity and allergic reactions are sometimes present from the adverse effects of antibiotics on the host (Nebedum J *et al*., 2009).

SSE have also attenuated the hyperthermia produced by 2,4-dinitrophenol (DNP). DNP is an inhibitor of ATP production in cell. It uncouples oxidative phosphorylation leading to release of energy as heat without generation of ATP which leads to increase in body temperature. Thus it's presumed that SSE also has its effect on oxidative phosphorylation.

It is also presumed that the presence of flavonoids may be contributing to antipyretic activity of ethanolic root extract. Thus, the present pharmacological evidence provides support for the folk fore claim as an antipyretic agent. Antipyretic activity along with antimicrobial potential clearly indicates therapeutic potential of *Sida spinosa* ethanolic extract.

Predicted mechanism of action of SSE



Further analyses are needed to determine the effect of SSE on electron transport chain and isolation of particular component.

ACKNOWLEDGMENT

With great reverence I wish to express my deepest thanks and appreciation to my esteemed guide Dr Preeti Kulkarni, Professor, Department of Pharmacology S.E.T's College of Pharmacy, Dharwad, for taking me under her leadership.

CONFLICT OF INTEREST

There is no conflict of interest associated with the authors of this paper.

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