Pharmacological evaluation of analgesic, anti-inflammatory and anti-pyretic activities of *amaranthus spinosus stem*.

Raj Kumar Singh Bharti[1], Sushil Kumar[2], Sateesh Kumar[3], Dinesh Kumar[4], Shivam*[5] and Amit Kumar[6]

[1] *Department of Pharmacology, School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Moradabad-244102, Uttar Pradesh, India*
[2] *Department of Pharmacology, School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Moradabad-244102, Uttar Pradesh, India*
[3] *Radha Govind College of Pharmacy, Near 2km RTO office Moradabad Uttar Pradesh, India*
[4] *Department of Pharmacology, School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Moradabad-244102, Uttar Pradesh, India*
[5] *Department of Pharmacology, School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Moradabad-244102, Uttar Pradesh, India*
[6] *Department of Pharmacology, School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Moradabad-244102, Uttar Pradesh, India*

[1] *rajroy.ars@gmail.com*
[2] *dr.sushiliftm@gmail.com*
[3] *kumarsatish86536@gmail.com*
[4] *dineshpharma181@gmail.com*
[5] *shivamdmohit@gmail.com*
[6] *amkm95461@gmail.com*

Corresponding Author: Raj kumar singh Bharti- rajroy.ars@gmail.com

Abstract

*The herbal medicines have stood the test of time for their safety, efficacy, lesser side effects, cultured and traditional acceptability. Natural plant-based product are promising for the drug discovery and they still continue to play a significant role in future drug development programs. Practitioners of herbal medicine generally use unpurified plant extracts containing several different constituents. The extracts of the plant (Stem) was evaluated for the different pharmacological activities. The need for safer and effective analgesic (*Acetic Acid Induced Writhing Test*, Eddy's Hot Plate, Tail Flick Test) anti-inflammatory (Rat hind paw edema test) and antipyretic agents and the lack of enough scientific data to support the claims made in ancient literature prompted the present study.*

Key words: Analgesic, Anti-pyretic and Anti-inflammatory, Plant Amaranthus *spinosus.*

1. Introduction

Pain involves a significant psychological component which can alter its perception. **[1]**.Inflammation and pain are most common aspects of human health. Instead improvements in our understanding of pathophysiological mechanisms of pain and inflammatory states, and the identification of multiple analgesic mechanisms, the clinical need for pharmacotherapy for painful conditions, that is effective and safe remains predominant **[2].** Treatment of inflammation is a debate as the conventional NSAIDS are commonest to cause Adverse Drug Reactions. Hence there is ongoing research to develop safer and more effective drugs for the therapy of inflammation [**19].** Fever is a complex physiologic response triggered by infectious or aseptic stimuli. Elevations in body temperature occur when concentrations of prostaglandin E_2 (PGE_2) increase within certain areas of the brain. These elevations alter the firing rate of neurons that control thermoregulation in the hypothalamus [**3].** *Amaranthus spinosus* are important for many pharmacological research and drug development. Due to large no of Pharmacology uses the *Amaranthus spinosus* are used as stomach ache, snake bites, control vomiting, antidote, acute bronchitis, diarrhoea, tooth ache, ulcerated mouth, eczema, burns, wounds, boils, gall bladder inflammation, arthritis, eyes wash etc. The present article is providing the pharmacological study on the plant (*Amaranthus spinosus*), traditional used and chemical constituents of the *Amaranthus spinosus* **[18].**

2. MATERIAL AND METHODS

2.1. Experimental animals

Animals will be fasted prior to test drug administration. For mice food was withdrawn 4 hours prior to drug administration. Following the period of fasting animals was weighed and then the test substance administered in a single dose of 2000 mg/kg to animals by oral gavages. After the test drug administration, food was withheld for next 3-4 hours. Following administration, the individually animals were closely observation for next 4 hours to see any clinical symptom, any change in behavior or mortality. After 6 hours of test administration the animals weighed again recorded. A careful clinical examination was made once in each day for next 14 days. Dosing continues depending on the fixed-time interval outcomes of all the animals up to that time. The testing stops when one of the following stopping criteria first is method: (a) 3 consecutive animals survive at the upper bound; (b) 5 reversals occur in any 6 consecutive animals tested; (c) At least 4 animals have followed the first reversal and the specified likelihood-ratios exceed the critical value. At last, the 10% of maximum dose will be considered safe to carry out the research work **[4].**

2.2. Grouping of Animals

Group I- Control group.
Group II- Standard drug group.
Group III- Test group low dose (EEAS)
Group IV- Test group high dose (MEAS).

2.3. Toxicity Study- For the assessment of Analgesic, Anti-inflammatory and Antipyretic activities, dose level was chosen in such a way that, dose was approximately one tenth (low

dose) and one fifth (high dose) of the maximum dose during acute toxicity studies (200 and 400 mg/kg/day). Diclofenac sodium was used as the reference drug for evaluation of the analgesic, anti-inflammatory and antipyretic activities.

2.4. Drugs and chemicals-

Drugs- Amaranthus *spinosus*

- Diclofenac Sodium (Novartis India Ltd.)
- Acetic acid (Otto Chemie Pvt Ltd, Mumbai)
- Carboxymethyl cellulose (SD fine chemicals)
- Sterile water for injection (Nirlife Health Care, Mumbai)
- Petroleum ether (SD fine chemicals)
- Ethyl alcohol
- Methanol
- Hydrochloric acid (SD fine chemicals)
- Sodium hydroxide (CDH Ltd, New Delhi)
- Chloroform (CDH Ltd, New Delhi)
- Glacial acetic acid
- Sulphuric acid (CDH Ltd, New Delhi)
- n- butanol
- α- napthol
- Lead acetate

3. Collection, identification and authentication of plant material-

The dried stem material was defatted with petroleum ether (60-80) and then extracted with Ethanol (95%, v/v) and Soxhlet apparatus. The extracts was filtered and Concentrated by distilling off the solvents and evaporated to dryness using rotatory vacuum evaporator.

3.1. Preparation of extracts- The dried stems materials was powdered and passed through a 20-mesh sieve. The stem material was defatted with petroleum ether (60-80) and then extracted with Ethanol (95%, v/v) and Soxhlet apparatus. The extracts was filtered and Concentrated by distilling off the solvents and evaporated to dryness using rotatory vacuum evaporator.

4. Experimental design

4.1. Assessment of analgesic activity: Animals were treated with test and standard drugs for 7 successive days once a day and test was performed on 7th day after 60 min administration of test drugs per oral and 30 min after standard drugs administration by i.p.

4.2. Acetic acid induced writhing test: The writhing test was performed as described by Kostar **[12].** After 30 min of treatment, each rates of each group was administered intra peritoneal with 0.6% acetic acid in normal saline at the dose 10 ml/kg. The rates were observed and counted for the number of abdominal constrictions and stretching's in a period of 0-20 min. A reduction in the writhing number compared to control group was evaluated for analgesia which was expressed as % inhibition of writhing

4.3. **Eddy's hot plate test:** The Hot plate test used to evaluates the thermal pain reflexes due to foot pad contact with a heated surface. Albino rats were divided into 6 groups each consisting of six animals. Group I served as vehicle control. Group II serves as standard drug and Group III and IV received EEAS (200 and 400mg/kg). Group V and VI received of MEAS (400 and 400mg/kg). All the doses were administered orally. After 30 min of treatment the rats were placed on a hot plate (55ºC) and the time interval between the placement of the animals and the occurrence of licking or shaking the hind paws was recorded as reaction time. The cut off time was set as 30 seconds **[13].**

4.4. **Tail flick method:** Prior to the analgesic experiments, the animals were screened for a sensitivity test by immersing the tip of tail (5 cm) gently in hot water (55^0C).Within a few seconds, the rats react by withdrawing tail. The reaction time is recorded by stopwatch. The reaction time was determined periodically after administration of the drugs. The cut off time of tail immersion was taken 15 seconds [**14].** Treatment and grouping were same as writhing test.

4.5. **Assesent of anti-inflammatory activity:** Animals were treated with test and standard drugs for 7 successive days once a day and test was preformed or 7th day after 60 min administration of test drugs per oral and 30 min after standard drugs administration.

4.6. **Carrageenan induced paw edema in rats:** This method was performed as previously described by **(Winter *et al.,* 1962).** After 60 min test and standard drugs each rat in all groups was subcutaneously injected with 0.1 ml of 1% (w/v) carrageenan in normal saline into sub plantar region of the right hind paw. The volume of right hind paw was measured at 1, 2 and 3 h after carrageenan injection and the edema volume was determined. The data were expressed as percentage of swelling compared with initial hind paw volume of each rat.

4.7. **Assesent of anti-pyretic:** Animals were treated with test and standard drugs for 7 successive days once a day and test was performed on 7th day after 60 min administration of test and standard drugs per oral. On 6th day pyrexia was induced with brewer's yeast and after 18 hr (7th day) temperature was noted down

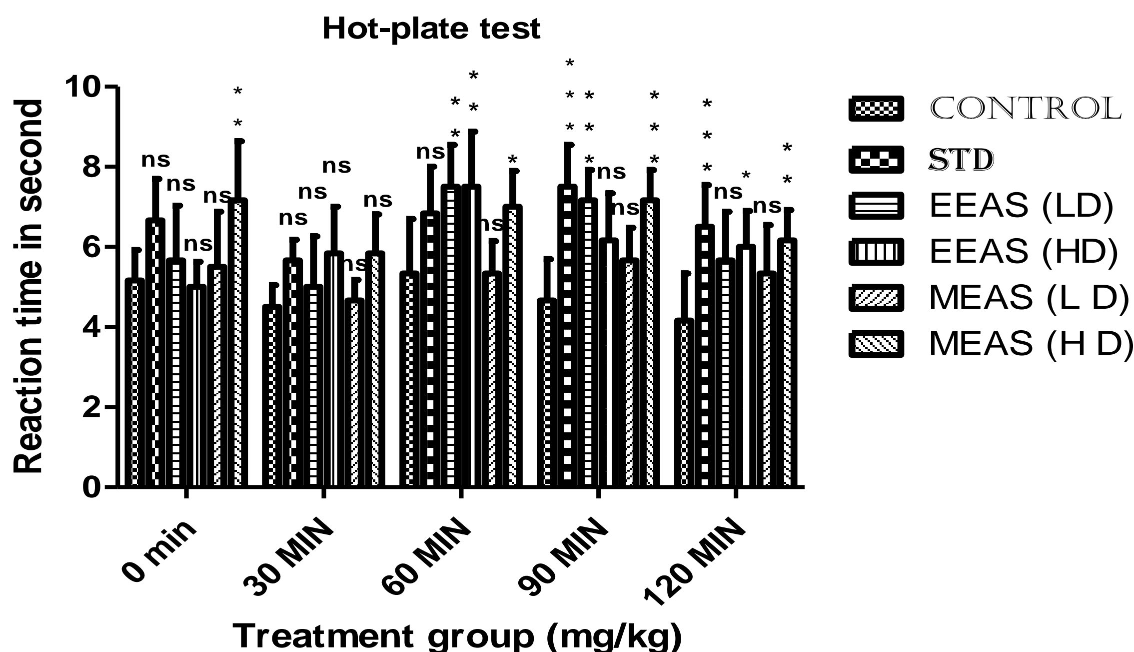
4.8. **Induction of yeast induced pyrexia:** Yeast induced pyrexia was used to evaluate the antipyretic activity of the test compounds. The body temperature of each rat was recorded by measuring the rectal temperature at predetermined time intervals. Fever was induced by induced by injecting 15% suspension of brewer's yeast following a standard method. The rats were allowed to remain quite in the case for some time. The mister probe was inserted 3-4 cm deep in to the rectal after fastening the tail recorded the basal rectal temperature. The animal where then given a subcutaneous injection of 10ml/kg of 15%w/v brewer's yeast suspended in 0.5%w/v CMC solution and the animal where returned to their housing case. 19 hr after yeast injection, the rats where again restrained in individual case to record their rectal temperature. Immediately the test compounds and standard, where administered orally at their respected doses. Rectal temperature of all the rats was recorded at 19 hr immediately before the administration of test compound, vehicle and paracetamol (150mg/kg, i.p) and again at 1 hr intervals up to three hr after the administration [**10].**

4.9. **Statistical analysis:** All the values were expressed as Mean ± S.E.M. the results were analyzed statistically by one-way ANOVA followed by Dunett's multiple comparison test, P<0.05 was considered significant when compared the control group.

5. RESULTS

5.1. Analgesic activity for EEAS and MEAS

5.1.1. Hot-Plate Test



5.1.2. Effects of EEAS and MEAS in hot plate test

The results showed that the reference drug Diclofenac (10 mg/kg) more significant increased the reaction time in rats at all the time intervals measured 90 and 120 min and non-significant 30min and 60 min. The EEAS (200 mg/kg) produced non-significant at 30 min and 120 min, but produced more significant effects at 90 min where as moderate 60 min. The EEAS (400 mg/kg) significant increased reaction time after 120 min and moderate significant at 60 min administration of the drugs and produced non-significant response at different time interval that is 30 and 90 minutes. The MEAS (200 mg/kg) produced non-significant effects after 30, 60, 90, 120 minutes. The MEAS (400 mg/kg) significant increased reaction time response after 60 min and non-significant effect at 30 min but moderate significant at 120 min administration of the drugs and also produced more significant response at different time intervals that is 90 minutes, EEAS and MEAS produced significant effect when compared to the control group.

5.1.3. Effects of EEAS and MEAS in Hot-Plate Test

S. No.	Treatment group	Dose (mg/kg)	Basal Reaction Time (Sec).	Reaction Time (sec).			
				30 min.	60 min.	90 min.	120 min.
1.	Control	10ml/kg	5.16±0.75	4.50±0.54	5.33±1.36	4.66±1.03	4.16±1.16
2.	Diclofenac sodium.	10	6.66±1.03^{ns}	5.66±0.51^{ns}	6.83±1.16^{ns}	7.50±1.04***	6.50±1.04***
3.	EEAS	200	5.66±1.36^{ns}	5.00±1.26^{ns}	7.50±1.04**	7.16±0.75***	5.66±1.21^{ns}
4.	EEAS	400	5.00±0.63^{ns}	5.83±1.16^{ns}	7.50±1.37**	6.16±1.16^{ns}	6.00±0.89*
5	MEAS	200	5.50±1.37^{ns}	4.66±0.51^{ns}	5.33±0.81^{ns}	5.66±0.81^{ns}	5.33±1.21^{ns}
6	MEAS	400	7.16±1.47	5..83±0.98^{ns}	7.00±0.89*	7.16±0.75***	6.16±0.75**

All values are expressed as Mean ± SEM, test employed ANOVA one way followed by Dunett's test (n=6); significant different from the control at *(P<0.05), ** (P<0.01), ***(P<0.001) and ns (non–significant) when compared to control group.

5.2. Anti-inflammatory activity for EEAS and MEAS

5.2.1. Carrageenan induced paw edema Test

5.2.2. Effect of EEAS and MEAS on Carrageenan induced paw edema

The anti-inflammatory effect of EEAS, MEAS and Diclofenac sodium on Carrageenan induced hind paw edema showed in table no 5.17. The reference drug Diclofenac sodium (10 mg/kg) produced more significant effects against Carrageenan induced inflammation after 1 hr, 2 hrs and 3 hrs of the administration. The dose of Diclofenac sodium (10mg/kg) exhibited more significant inhibition of 71.08% after 2 hrs, the effect increased at 3 hrs that is 81.39%.The EEAS (200mg/kg) exhibited more significant inhibition of 45.88 % after 2 hrs, the effect increased at 3 hrs that is 34.88 %. The EEAS (400mg/kg) exhibited more significant inhibition of 97.64 % after 3 hrs, the effect decrease at 2 hrs that is 96.51 %. The MEAS (200 mg/kg) produced moderate significant effect after 3 hr and produced more significant effects after 1 hr that is (17.44 %) and (32.53 %) respectively whereas after 2 hrs produced no significant effect 11.76%. MEAS (400 mg/kg) produced more significant effect after 1 hr, 2 hrs and 3 hrs and exhibited more significant inhibition at 2 hrs that is 67.24% and at 3 hrs that is 76.31 when compared with control group.

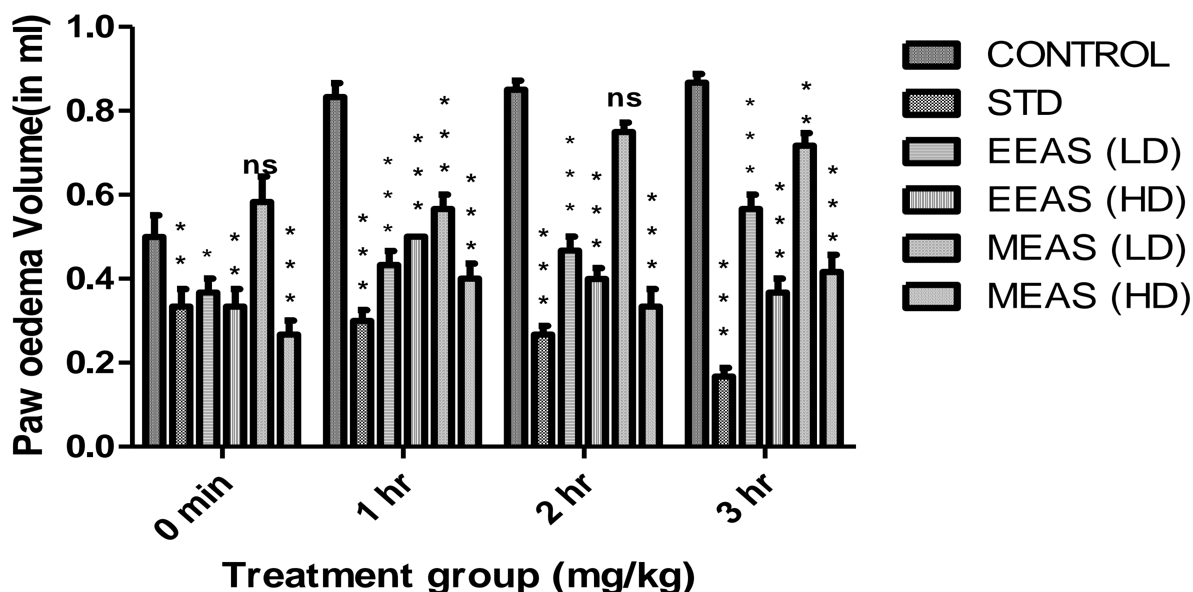


Figure: Effect of EEAS and MEAS in Carrageenan induced paw edema

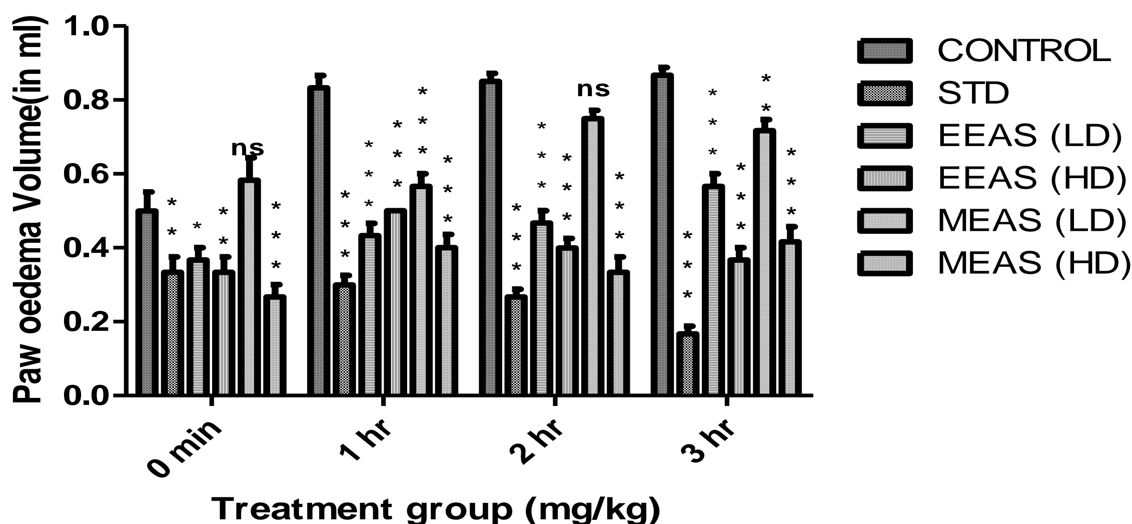
5.2.3. Effect of EEAS and MEAS on Carrageenan induced paw edema

S. No.	Treatment group	Dose (mg/kg)	(Paw size) Paw Volume in (ml) at hr.			
			Initial volume	1 hr	2 hr	3hr
1.	Control	10 ml/kg	0.50±0.05	0.83±0.03	0.85±0.02	0.86±0.21
2.	Diclofenac Sodium	10	0.33±0.04	0.30±0.02***	0.26±0.02***	0.16±0.02***
3.	EEAS	200	0.36±0.03	0.43±0.03^{ns}	0.46±0.03***	0.56±0.03***
4.	EEAS	400	0.04±0.58	0.00±0.56***	0.02±0.75***	0.03±0.71***
5.	MEAS	200	0.58±0.06	0.56±0.03***	0.75±0.02^{ns}	0.71±0.03**
6.	MEAS	400	0.26±0.03	0.40±0.03***	0.33±0.04***	0.41±0.04***

All values are expressed as Mean ± SEM, test employed ANOVA one way followed by Dunett's test (n=6); significant different from the control at *(P<0.05), ** (P<0.01), ***(P<0.001) and ns (non–significant) when compared to control group.

5.2.4. % inhibition for effect of EEAS and MEAS on Carrageenan induced paw edema

S. No.	Treatment group	Dose (mg/kg)	% inhibition		
			1 hr	2hr	3 hr
1.	Control	10 ml/kg	–	–	–
2.	Diclofenac sodium	10	63.85	71.08	81.39
3.	EEAS	200	48.19	45.88	34.88
4.	EEAS	400	100	97.64	96.51
5.	MEAS	200	32.53	11.76	17.44
6	MEAS	400	51.80	61.17	52.32



Effect of EEAS and MEAS in Carrageenan induced paw edema

5.3. Anti-pyretic activity for EEAS and MEAS

5.3.1. Brewer's yeast induced pyrexia test

5.3.2. Effect of EEAS and MEAS on Brewer's yeast induced pyrexia test

In this test EEAS (200mg/kg) produced significant effect after 1 hr administration and after 2 hrs produced moderate significant whereas after 3 hr and 4 hr more significant effect respectively. EEAS (400 mg/kg) produced moderate significant effects after 1 hr and more significant after 2 hrs, 3 hrs and 4 hrs of drug administration. MEAS (200 mg/kg) produced non- significant effect after 1 hr and 2 hr but produced more significant effect after 3 hrs and

4 hrs of drug administration. MEAS (400 mg/kg) produced moderate significant effect after 2 hr while more significant effect after 3 hrs and 4 hrs of drug administration. The EEAS and MEAS (200 and 400 mg/kg) and Diclofenac sodium (10 mg/kg) decreased the rectal temperature at different time interval that is after 1 hr, 2 hrs, 3 hr and 4 hrs administration of the drug. The standard drug Diclofenac (10 mg/kg) showed more significant effect when compared to the control group.

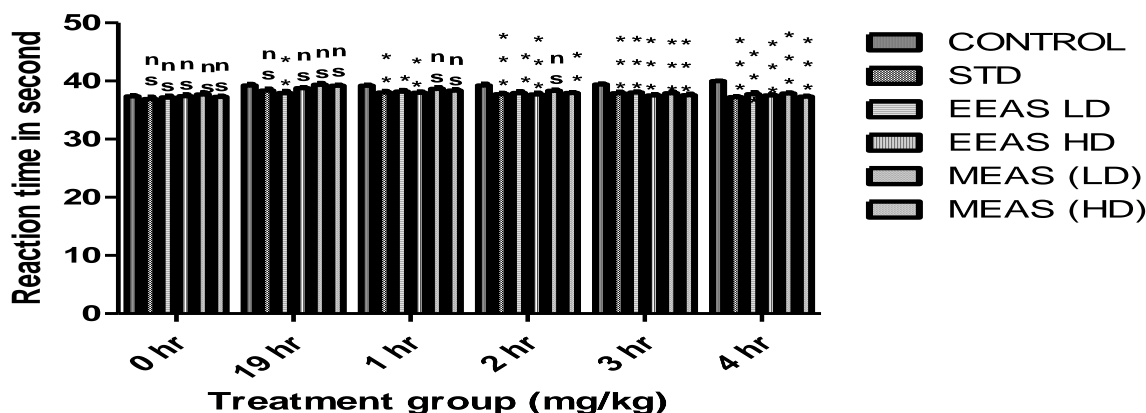


Figure: Effect of EEAS and MEAS on Brewer's yeast induced pyrexia test

5.5.3. Effect of EEAS and MEAS on Brewer's yeast induced pyrexia test

S.No.	Treatment group	Dose (mg/kg)	Initial Temp. (^{0}C).	Temp. after 19 hr of yeast admin.	Rectal temperature after yeast admin.			
					1 hr	2hr	3 hr	4 hr
1.	Control	10 ml/kg	37.33± 0.19	39.23± 0.24	39.18± 0.18	39.21± 0.28	39.40± 0.16	39.96±0.10
2.	Diclofenac sodium	10	36.88± 0.39	38.36±0.34^{ns}	38.01±0.24**	37.71±0.21***	37.88±0.25***	37.21±0.12***
3.	EEAS	200	37.18± 0.27	37.95±0.31^{ns}	38.20±0.23*	37.91±0.32**	38.00±0.20***	37.73±0.31***
4.	EEAS	400	37.38± 0.30	38.76±0.18^{ns}	37.98±0.20**	37.68±0.29***	37.50±0.22***	37.50±0.18***
5	MEAS	200	37.66± 0.34	39.36±0.29^{ns}	38.65±0.30^{ns}	38.30±0.21^{ns}	37.90±0.26***	37.85±0.17***
6	MEAS	400	37.25± 0.17	39.20±0.17^{ns}	38.36±0.19^{ns}	37.91±0.16**	37.58±0.21***	37.30±0.15***

All values are expressed as Mean ± SEM, test employed ANOVA one way followed by Dunett's test (n=6); significant different from the control at *(P<0.05), ** (P<0.01), *** (P<0.001) and ns (non–significant) when compared to control group.

6. Discussion

In the present study, an attempt was to investigate analgesic, anti-inflammatory and antipyretic potential of EEAS and MEAS (200mg/kg and 400mg/kg). Pain is the most common reason people seek medical attention. It can be defined simply as an undesirable physical experience and it can be classified as acute or chronic. Treatment for chronic pain is a major public health problem due to the recurrent use of available drugs that have undesirable side effect **[5].** The MEAS (200 mg/ kg) also produced non-significant effects and exhibited 11.06% inhibition of writhing in rats, whereas MEAS (400 mg/kg) produced moderate significant effects and also exhibited 23.17% inhibition of writhing in rats. In this study the reference drug Diclofenac sodium (10 mg/kg) more significantly decreased the number of writhing and also exhibited the 72.32% inhibition in writhing in rats. The stem extracts of the Amaranthus *spinosus* (EEAS and MEAS) and Diclofenac sodium (10 mg/kg) also presented a longer latency time than the control group in the hot plate test in a dose related manner.

The results showed that the reference drug Diclofenac (10 mg/kg) more significant increased the pain latency in rats at 90 min and moderate significant at 120 min where as non-significant effect after 30 and 60 min of treatment. The EEAS (200 mg/kg) produced non-significant nociceptive response at different time measured 0, 30 and at 120 min and moderate significant at 60 min where as more significant at 90 min. The EEAS (400 mg/kg) significantly increased latency of nociceptive response after 120 min administration of the drugs and produced non-significant response at different time interval that is 30 and 90 minutes where as moderate significant at 60 min. The MEAS (200 mg/kg) produced non-significant nociceptive response after 30 min 60 min, 90 min and 120 min. The MEAS (400 mg/kg) significant increased latency of nociceptive response after 60 min and non-significant at 30 min after the administration of the drugs and also produced more significant response at different time intervals that is 90 min where as moderate at 120 min. The Diclofenac (10 mg/kg) more significant increased the pain latency in rats at all the time intervals measured 90 and 120 min and non-significant at 30 min and 60 min. The hot plat method is considered to be selective for the drugs acting centrally. The hot plat test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity **[6].** It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally [**7].** Therefore, the ethanolic and methanolic extracts of the amaranthus spinosus may possess central activity. Again, narcotic analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain **[8].** The ethanolic and methanolic extract of *Amaranthus spinosus* (EEAS and MEAS) were also evaluated in the tail immersion test for its analgesic activity. This method is surpassingly mediated and has selectivity for centrally acting analgesics **[9].** All results obtained from writhing, hot plate and tail flick tests used in

this study indicated that the EEAS and MEAS both the drugs possessed analgesic activity. The analgesic mechanisms of the EEAS and MEAS both the drugs may be centrally mediated. The inflammatory mechanisms of the EEAS and MEAS both the drugs may be peripherally mediated. The results obtained from the carrageenan induced paw edema test used in the present study indicated that the both test (EEAS and MEAS) drugs possess anti-inflammatory activity. The present finding of the study indicates that the EEAS and MEAS may be centrally acting. Fever is defined as the elevation of core body temperature above normal; in normal adults, the average oral temperature is 37.6°C (98.6°F) **[16].** Pyrexia is caused as a result of infection, tissue damage, inflammation, graft rejection, malignancy or due to microbial infections such as bacteria or viruses triggered the body's defense mechanisms. Normally, the infected tissue initiates the synthesis of pro-inflammatory mediator viz., prostaglandin E2 (PGE2) **[15].** The results in the present study showed that the EEAS and MEAS possessed the significant anti-pyretic effect in yeast-elevation of the body temperature in rats where as less effective when compared Diclofenac sodium (10 mg/kg). From the study it may also be said that traditional uses of amaranthus spinosus for the treatment of type of pain and fever conditions has got definite basis. However further investigations are required to identify the active constituent and to verify the therapeutic merits of the active constituent **[17]**.

7. Conclusion

The results obtained from the in-vivo animal studies indicate that the extract of Amaranthus *Spinosus* (EEAS and MEAS) possesses considerable analgesic, anti-inflammatory and antipyretic activities but is less potent than the reference drugs. However, further studies are required to elucidate the exact mechanism of the analgesic, anti-inflammatory and antipyretic activities as well as establish their efficacy and safety for clinical purpose.

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