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Syringaldehyde demonstrates analgesic, anti-nociceptive and anti-hyperalgesic potency in rodent pain models

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ABSTRACT

Discovery of novel treatments for management of various pain conditions is an unmet need. Syringaldehyde is a phytochemical compound with anti-inflammatory, anti-oxidant potential. In this study, experimental animal models of analgesia, nociception and inflammatory pain were utilized to ascertain pharmacological potency of Syringaldehyde. Syringaldehyde was tested in mouse tail flick test and formalin-induced nociceptive pain. To assess the pharmacological effect of Syringaldehyde, mouse models of tail flick test and formalin- induced nociception were used. To assess effect of Syringaldehyde in inflammatory pain, Carrageenan induced thermal pain hypersensitivity (hyperalgesia) and complete Freund's adjuvant (CFA) induced mechanical pain hypersensitivity (hyperalgesia) in rats was used along with analysis of expression levels of inflammatory mediators. Dose-dependent analgesic effect was seen with Syringaldehyde treatment in tail flick test, while Syringaldehyde pretreatment substantially reduced formalin-induced nociceptive pain in second phase of formalin test. In inflammatory pain induced with intraplantar injection of Carrageenan and CFA, Syringaldehyde treatment reversed thermal and mechanical hyperalgesia with inhibition of elevated inflammatory pain mediator levels. In this study, Syringaldehyde demonstrated analgesic, anti-nociceptive activity and inhibited inflammatory pain through modulation of inflammatory mediators.

KEYWORDS: Syringaldehyde, Carrageenan, CFA, Analgesic, Anti-nociceptive, Inflammatory pain

INTRODUCTION

Pain is an unpleasant feeling triggered by tissue damage and is the most common ailment affecting patients suffering from conditions like traumatic accidental injuries, chronic diseases like cancer, diabetes (Walters and Williams, 2019). Chronic pain because of underlying tissue damage as well as inflammation leads to diminished quality of life, lower productivity in affected patients (Kawai et al., 2017). Opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are regularly being used for treatment of chronic painful conditions (Patel et al., 2017; Miranda et al., 2019). However, effectiveness of opioids and NSAID treatments remain inadequate as long-term use of NSAIDs is associated with gastrointestinal disturbances like ulcers, lesions (Wang et al., 2014) and chronic use of opioids leads to adverse effects like development of tolerance, respiratory depression (Becker 2010; Sneddon 2018). Thus for better management of acute and chronic pain ailments, discovery of novel pain therapies with better pharmacological efficacy is necessary.

Syringaldehyde (3,5-dimethoxy-4-hydroxybenzaldehyde) is a low molecular weight phenolic aldehyde found in various plants like Magnolia officinalis (Shen et al., 2009). There are number of pharmacological properties that Syringaldehyde is known to exhibit, including anti-inflammatory, anti-oxidant, cardioprotective, neuroprotective and anti-diabetic activity (Stanikunaite et al., 2009; Boxkurt et al., 2014; Wu et al., 2022).

In order to ascertain pharmacological acitivity of Syringaldehyde in pain, this study employed the following methods: the mouse tail flick and formalin test, Carrageenan- and CFA-induced inflammatory pain in rats. Furthermore, expression levels of inflammatory pain mediators was also measured to correlate their role in Carrageenan- and CFA-induced inflammatory pain.

MATERIALS AND METHODS

Animals

Animal house of Dayananda Sagar University (Bengaluru, India) supplied adult male C57BL6 mice (22-25 g, 7-8 weeks old) and male Sprague-Dawley rats (180- 200 g, 7-8 weeks old). All animals were maintained under controlled conditions like 12-h light-dark cycle and free access to food, water. Before experimental activity, animals were acclimatized to laboratory conditions for one week. Dayananda Sagar University's Institutional Animal Ethics Committee (IAEC) approved the experimental methods and procedures in animals.

Compounds and treatment

Syringaldehyde, Naproxen, λ-carrageenan and CFA were procured from Sigma (Sigma, India), formaldehyde (formalin) was procured from Qualigens. Oral administration of Syringaldehyde (10, 30, 100 mg/kg) and Naproxen (150 mg/kg) was done in mice for tail flick test and formalin test. Similarly, Syringaldehyde (5, 15, 50 mg/kg, po) and Naproxen (20 mg/kg) were given orally to rats in inflammatory pain models induced by Carrageenan and CFA.

Tail flick test

Tail flick latencies were recorded by applying heat stimulus to mouse tail using tail flick apparatus (Ugo Basile) and time duration (in seconds) taken by mouse from application of heat stimulus to withdrawal of tail was recorded as tail-flick latency. Mouse tail was removed from heat stimulus in absence of tail withdrawal after 15 seconds (cut-off time) to avoid possible tissue damage (Kalange et al., 2007). Baseline tail flick latencies were recorded, mice were divided into 5 groups; vehicle control, Syringaldehyde (10, 30, 100 mg/kg) and Naproxen (150 mg/kg). Tail flick latencies were measured in mice after 30 min, 1 h, 2 h, 4 h

of treatment. Analgesic effect was quantified as percentage maximum possible effect (%MPE) using following formula (Matsumoto et al., 2004)-

% MPE= (100 x (post-treatment latency - baseline latency)/ (15 - baseline latency))

Formalin test

Formalin test was done as per procedure detailed in earlier literature reports (Dubuisson and Dennis 1977; Zhang et al., 2018). Treatment groups consisted of vehicle control, Syringaldehyde (10, 30, 100 mg/kg) and Naproxen (150 mg/kg) in mice. Intraplantar subcutaneous injections of formalin (20 µl/ mouse, 2.5% v/v in saline) in hind paw were done 1 h of treatment. Duration of licking and biting events was recorded as a measure of nociceptive pain for 40 min after formalin injection. Nociceptive pain for first 5 min is considered as acute first phase and nociceptive pain for 16-40 min is considered as chronic second phase. Percentage inhibition for first and second phase was calculated using following formula-

% Inhibition= (100 – ((treatment reading/average vehicle reading) x 100))

Carrageenan induced inflammatory hyperalgesia

Hargreaves method (Hargreaves et al., 1988) was used to determine baseline paw withdrawal latency (PWL) of rats. Using Plantar test apparatus (Ugo Basile), heat stimulus was applied to rat hindpaw and time duration (in seconds) taken by rat to withdraw hindpaw was recorded as PWL. Heat stimulus application was stopped if rat did not withdraw hindpaw after 20 seconds (cut-off time) to avoid possible tissue damage. Baseline PWL was recorded and rats were distributed into 5 groups; vehicle control, Syringaldehyde (5, 15, 50 mg/kg) and Naproxen (20 mg/kg). Rats received λ-carrageenan (100 µl per rat, 1% in saline) in plantar surface of hindpaw at one hour after treatment. PWL was measured at 1, 3, 6 h of

Carrageenan injection (Zhu et al., 2012) to ascertain effect of Syringaldehyde and Naproxen treatment. Analgesic effect was determined as % MPE using formula mentioned below:

% MPE= (post-treatment PWL – vehicle PWL)/(20 - vehicle PWL) x 100

CFA induced inflammatory hyperalgesia

A set of 8 von Frey filaments (Bioseb) ranging from 0.4 g to 15 g was used to measure mechanical paw withdrawal threshold (PWT) in rats by up-down technique (Chaplan et al., 1994). Baseline PWT was recorded and CFA (50 µg per 100 µl per rat) was injected into plantar surface of rat hindpaw for induction of inflammatory pain. At 24 h post-CFA injection, development of inflammatory hyperalgesia was assessed (Munro et al., 2011; Larsen et al., 2016) by recording mechanical PWT. Rats with mechanical hyperalgesia were divided into 5 groups; vehicle control, Syringaldehyde (5, 15, 50 mg/kg) and Naproxen (20 mg/kg). One-hour after oral administration, effect of treatment was assessed by recording mechanical PWT. In order to ascertain anti-hyperalgesic effect %MPE was determined using formula:

%MPE= (post-treatment PWT – vehicle PWT)/(15 - vehicle PWT) x 100

mRNA analysis

COX-2 and PGE2 mRNA expression levels were measured in Carrageenan and CFA injected hindpaw samples using reverse transcription quantitative polymerase chain reaction (RT-PCR) assay. After recording of PWL/ PWT, animals were sacrificed and ipsilateral (Carrageenan/ CFA injected) hindpaws were collected to assess mRNA expression. Contralateral (Carrageenan/ CFA un-injected) hindpaws from vehicle group were collected and used as normal control paws. Hind paw samples were minced in presence of liquid nitrogen, total RNA was extracted using RNA extraction kit (Krishgen Biosystems). After

measuring RNA concentration and purity, isolated RNA was reverse transcribed into single stranded cDNA using iScript cDNA synthesis kit (Bio-Rad). RT-PCR was performed with SYBR green (Bio-Rad) using RT-PCR detection system (Applied Biosystems). Gene expression levels of inflammatory mediators was in test samples was normalized with GAPDH (housekeeping gene) and relative fold over gene expression was calculated as - Relative expression= 2^-(Ct GAPDH – Ct target gene)

Primers used for gene expression are

- GADPH (Saliba et al., 2018)
    - F- 5'- ATGCTGGTGCTGAGTATGTC -3' &
    - R- 5'- AGTTGTCATATTTCTCGTGGGTT -3'
- COX-2 (Saliba et al., 2018)
    - F- 5'- GGCTTACAAGACGCCACATCACCT -3' &
    - R- 5'- TGGTTTAGGCGGCCGGGGAT -3'
- PGE2 (Sun et al., 2018)
    - F- 5'- CCCACTCACCTGCTGCTACTC-3' &
    - R- 5'- AGAAGTGCTTGAGGTGGTTGTG-3'

Statistical analysis

To analyse statistical outcomes of study, 2-way analysis of variance (ANOVA) was performed with Bonferroni post-test for tail flick test, nociception in formalin test, PWL in Carrageenan test. Whereas, 1-way ANOVA was performed with Dunnett's post-test to analyse percentage inhibition in both phases of formalin test, % MPE and mRNA expression in Carrageenan and CFA test. Data is represented as average mean with standard error of mean (SEM) and p value less than 0.05 was considered as statistically significant.

RESULTS

Effect of Syringaldehyde in Tail flick test

This study involved measuring baseline tail flick latencies in mice followed by treatment with Syringaldehyde (10, 30, 100 mg/kg, po) or Naproxen (150 mg/kg, po). Treatment with Syringaldehyde exhibited dose-dependent analgesic activity as seen by increase in tail flick latencies (Table 1). Syringaldehyde (100 mg/kg) showed analgesic effect at 1 h (52% MPE, $P<0.001$) and 2 h (35% MPE, $P<0.01$) post-treatment when compared to vehicle control (figure 1). At 30 minutes (30% MPE, $P<0.05$), 1 h (75% MPE, $P<0.001$), 2 h (60% MPE, $P<0.001$) analgesic effect of Naproxen (150 mg/kg) was seen (figure 1).

Table 1

Formalin test

Biphasic pattern of nociceptive pain consisting of acute first phase and chronic second phase was seen with injection of formalin in mouse hindpaw. In first phase, increased paw licking and biting duration was observed immediately after injection of formalin that lasted for about 5 minutes. The intermediate phase with minimal nociceptive behavior was followed by second phase characterized by increased paw licking and biting duration from 16-40 min (Table 2). Treatment with Syringaldehyde had no effect on paw licking and biting behavior in first phase, while in second phase Syringaldehyde (30 and 100 mg/kg) dose-dependently decreased cumulative paw licking and biting behavior (Table 2) in second phase by 39% ($P<0.05$) and 62% ($P<0.001$, figure 2B) respectively. Treatment with Naproxen inhibited nociceptive pain in first phase (38%, $P<0.01$, figure 2A) and second phase (66%, $P<0.001$, figure 2B).

Table 2

Carrageenan induced inflammatory pain

Baseline PWL of rats was recorded and subsequently decrease in PWL was observed with intraplantar injection of Carrageenan indicating development of hyperalgesia in vehicle control group and maximal thermal hyperalgesia was seen at 3 h post-injection (baseline PWL 10.55 s versus 3 h PWL 7.06 s, Table 3) and hyperlgesia persisted till 6 h. When compared to vehicle group, treatment with Syringaldehyde increased PWL (Table 3). Syringaldehyde (15 and 50 mg/kg) showed analgesic effect with 31% MPE ($P<0.05$) and 56% MPE ($P<0.001$) respectively at 3 h post-Carrageenan injection (fig. 3A). Naproxen, too, reversed carrageenan induced thermal hyperalgesia and demonstrated 37 % MPE at 1 h ($P<0.01$) and 67% MPE at 3 h ($P<0.001$, fig. 3A). Carrageenan injection in ipsilateral hind paw caused increase in mRNA expression of COX-2 (5.7 fold, fig. 3B) and PGE2 (8.9 fold, fig. 3C) when compared to contralateral hind paw. Syringaldehyde treatment (5, 15, 50 mg/kg) decreased expression level of COX-2 mRNA (4.6, 3.9, 3.5 fold, fig. 3B) and PGE2 mRNA (7.9, 7.8 and 4.4 fold respectively, fig. 3C) with 50 mg/kg dose showing statistically significant reversal of PGE2 mRNA levels.

Table 3

CFA induced inflammatory pain

Using von Frey filament up-down method, baseline PWT of rats was recorded. Intraplantar injection of CFA in rat hind paw decreased PWT when compared baseline PWT indicating mechanical hyperalgesia at 24 h post-injection (baseline PWT 12.13 g versus 1.70 g, Table 4). Syringaldehyde treatment increased PWT (Table 4) and Syringaldehyde (15 and 50 mg/kg) showed anti-hyperalgesic effect of 18% MPE ($P<0.05$) and 48% MPE ($P<0.001$) respectively (fig. 4A). Naproxen attenuated CFA induced thermal hyperalgesia and demonstrated 57 % MPE post-treatment ($P<0.001$, fig. 4A). Injection of CFA increased mRNA expression of COX-2 (3.7 fold, fig. 4B) and PGE2 (15.36 fold, $P<0.001$, fig. 4C) in

ipsilateral hind paw as compared to contralateral hind paw. Treatment with Syringaldehyde (5, 15, 50 mg/kg) decreased COX-2 mRNA levels (3.96, 2.93, 2.76 fold respectively, fig. 4B) and PGE2 mRNA levels (10.91, 9.28 and 7.29 fold respectively, fig. 4D) with 50 mg/kg dose showing statistically significant revrsal of PGE2 mRNA ($P<0.01$).

Table 4

DISCUSSION

The results from this study have demonstrated that Syringaldehyde is effective in tail-flick test, formalin test, inflammatory pain induced by Carrageenan and CFA injection.

In tail-flick test, Syringaldehyde demonstrated substantial analgesic effect in mice at 1 h and 2 h post-treatment, with maximal analgesic effect seen at 1 h post-treatment. Tail flick test evaluates analgesic effect of compounds and responses in the tail flick test are mediated at the spinal level (Sora et al., 1997; Cavendish et al., 2015; Mischkowski et al., 2019; Singh et al., 2018). In this study, effect of Syringaldehyde may involve pain mechanisms at spinal level. As demonstrated in earlier reports (Zapata-Morales et al., 2016), Naproxen exhibited significant analgesia in tail flick test.

In formalin test, licking and biting duration of formalin-injected hindpaw is a robust readout of nociceptive pain (Hunskar and Hole 1987; Coderre et al., 1994; Meunier et al., 1998; Vidal-Torres et al., 1994). Injection of formalin in hindpaw caused biphasic nociceptive pain response with first phase lasting for about 5 minutes and inflammatory second phase seen from 16-40 minutes post formalin injection (Ahmadiani et al., 2000; López-Cano et al., 2017). Pain response in first phase of formalin test is due to increased activity of peripheral afferent C-fibers (Tjolsen et al., 1992), while in inflammatory second phase release of inflammatory pain mediators like serotonin, histamine, bradykinin, prostaglandins cause central sensitization of nociceptors (Hunskar and Hole 1987; Tjolsen et al., 1992; Shibata et al., 1989;

Basbaum et al., 2009). Lack of nociceptive pain during intermediate phase between phase 1 and phase 2 is due to transient inactivity in response to formalin induced nociception during first phase (Fu et al., 2000). Similarly, in this study, formalin injection caused biphasic nociceptive pain response characterized by increased paw licking and biting duration in first phase, followed by intermediate phase with minimal pain response and subsequently increased nociceptive pain response in second phase. Syringaldehyde treatment did not affect first phase, and dose dependently attenuated the nociceptive pain response in second phase. Anti-nociceptive effect of Syringaldehyde may be due inhibition of inflammatory pain mediators contributing to central sensitization. In agreement with previously reported literature studies (Zapata-Morales et al., 2016), Naproxen demonstrated anti-nociceptive effect in both acute first phase and chronic second phase.

Carrageenan induced inflammatory pain involves role of inflammatory mediators like histamine, serotonin, bradykinin and prostaglandins (Rauf et al., 2015). Carrageenan induced pain is categorized as early phase (1 h) and late phase (3-4 h), where late phase is characterized by COX-2 activation (Dirig et al., 1998; Chou et al., 2003). Consistent with previous studies (Zhang et al., 2002), in this study, intraplantar Carrageenan injection developed thermal hyperalgesia with maximal peak thermal hyperalgesia seen at 3 h post-injection. Syringaldehyde treatment dose-dependently reversed thermal hyperalgesia. In this study, injection of carrageenan caused increase in mRNA expression levels of COX-2 and PGE2 in ipsilateral paw. Higher expression levels of PGE2 followed by COX-2 induction is known to play a pivotal role in development of thermal hyperalgesia (Dirig et al., 1998; Nantel et al., 1999) whereas higher levels of PGE2 sensitizes peripheral pain nociceptors and cause hyperalgesia (Cunha et al., 1992; Ferreira et al., 1993). In this study, Carrageenan induced thermal hyperalgesia was observed with increased COX-2 and PGE2 mRNA levels, suggesting role of these inflammatory mediators in development of inflammatory

hyperalgesia. Syringaldehyde treatment attenuated hyperalgesia with reversal of increased COX-2 and PGE2 mRNA levels. The results indicate pharmacological effect of Syringaldehyde in attenuating Carrageenan-induced inflammatory pain by inhibiting COX-2 and PGE2 expression levels. Naproxen significantly reversed Carrageenan-induced thermal hyperalgesia and reversed COX-2 and PGE2 expression.

Intraplantar injection of CFA induces robust inflammatory pain characterised by mechanical hyperalgesia (Munro et al., 2011; Larsen et al., 2016; Biscaia at al., 2022), with increased expression levels of COX-2 and PGE2 (Hay et al., 1997; Yuan et al., 2018). Anti-inflammatory compound, Naproxen is known to attenuate inflammatory pain induced by CFA (Ahn et al., 2009). Local activation of COX-2 causes increase in PGE2 levels resulting in peripheral pain sensitization at site of injury (Broom et al., 2004). Similarly, in this study, CFA injection caused mechanical hyperalgesia with increased COX-2 and PGE2 mRNA levels. Furthermore, treatment with Syringaldehyde attenuated mechanical hyperalgesia and reversed COX-2 and PGE2 mRNA expression levels. These results suggest that anti-hyperalgesic effect of Syringaldehyde in CFA-induced inflammatory pain might be mediated by inhibition of COX-2 and PGE2 inflammatory mediators.

CONCLUSION:

This study has demonstrated pharmacological effect of Syringaldehyde in analgesic, antinociceptive and inflammatory pain models. Treatment with Syringaldehyde modulates expression of inflammatory pain mediators to exert its pharmacological effect in animal models of inflammatory pain. Data from this study, demonstrates potential of Syringaldehyde as a novel therapeutic option with pharmacological efficacy for treatment of pain and offers a promising path to further explore efficacy of Syringaldehyde in chronic pain models.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

Figure 1.

Effect of Syringaldehyde on tail flick test in mice (% MPE). Values (n=6) are expressed as mean±SEM. ***P<0.001, **P<0.01, *P<0.05 vs Vehicle Control, Two-way ANOVA followed by Bonferroni post-test

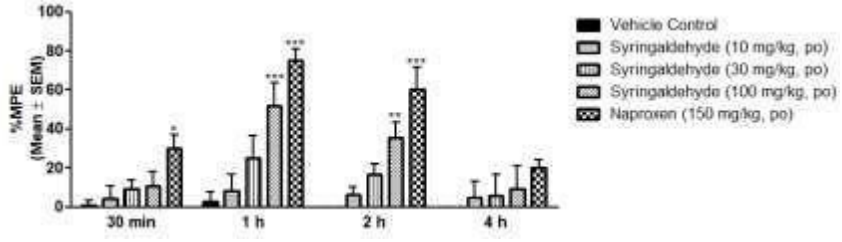


Figure 2. Effect of Syringaldehyde on formalin test in mice (% Inhibition: A- First phase, B- Second phase). Values (n=6) are expressed as mean±SEM. ***P<0.001, **P<0.01 vs Vehicle Control, One-way ANOVA followed by Dunnett's post-test

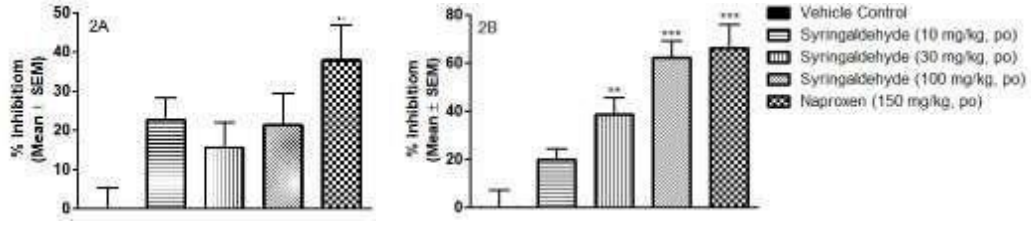


Figure 3. Effect of Syringaldehyde on Carrageenan induced inflammatory pain in rats (A- % MPE, B- COX-2 mRNA levels, C- PGE2 mRNA levels). Values (n=6) are expressed as mean±SEM. 3A: ***P<0.001, **P<0.01, *P<0.05 vs Vehicle Control, Two-way ANOVA followed by Bonferroni post-test; 3B, 3C: ***P<0.001, **P<0.01, *P<0.05, ##P<0.001, #P<0.05 vs Vehicle Control, One-way ANOVA followed by Dunnett's post-test

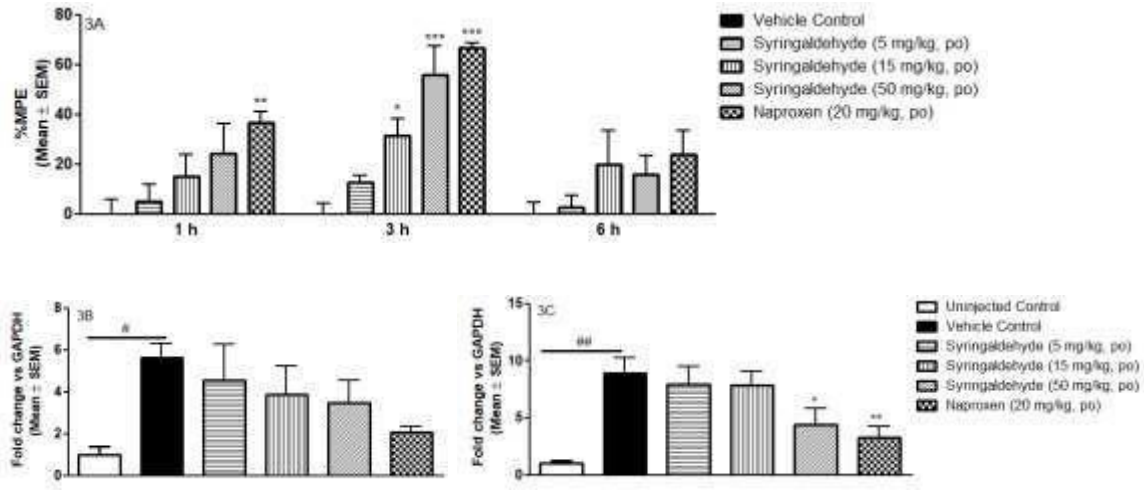
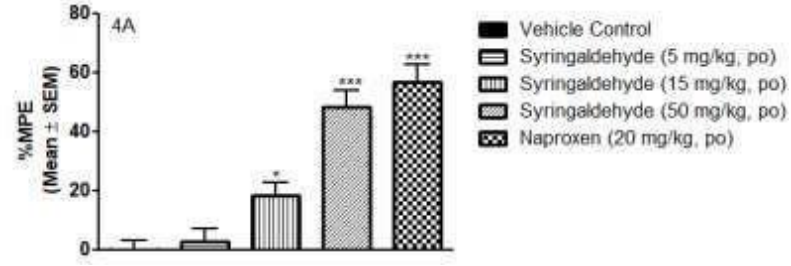
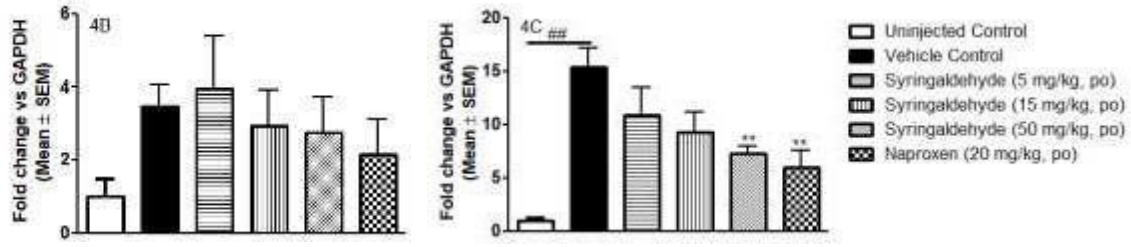


Figure 4. Effect of Syringaldehyde on CFA induced inflammatory pain in rats (A- % MPE, B- COX-2 mRNA levels, C- PGE2 mRNA levels) Values (n=6) are expressed as mean±SEM. ***P<0.001, **P<0.01, *P<0.05, ##P<0.001 vs Vehicle Control, One-way ANOVA followed by Dunnett's post-test





TABLES

Table 1. Effect of Syringaldehyde on tail flick test in mice

Treatment	Tail flick latencies (sec, Mean ± SEM)				
	Baseline	**30 minutes**	**1 h**	**2 h**	**4 h**
Vehicle Control	5.56 ± 0.36	5.64 ± 0.39	5.83 ± 0.49	5.28 ± 0.55	5.23 ± 0.43
Syringaldehyde 10 mg/kg	5.54 ± 0.35	5.89 ± 0.85	6.27 ± 0.90	6.16 ± 0.38	6.04 ± 0.79
Syringaldehyde 30 mg/kg	5.53 ± 0.31	6.40 ± 0.52	7.85 ± 1.17	7.17 ± 0.41	6.04 ± 1.14
Syringaldehyde 100 mg/kg	5.57 ± 0.30	6.60 ± 0.71	10.58 ± 1.02***	8.97 ± 0.72**	6.36 ± 1.31
Naproxen 150 mg/kg	5.63 ± 0.29	8.53 ± 0.48*	12.61 ± 0.58***	11.39 ± 0.97***	7.49 ± 0.54
***P<0.001, **P<0.01, *P<0.05 vs Vehicle Control, Two-way ANOVA followed by Bonferroni post-test					

Table 2. Effect of Syringaldehyde on formalin induced nociceptive pain in mice

Treatment	Nociceptive pain (sec, Mean ± SEM)							
	0-5 min	**6-10 min**	**11-15 min**	**16-20 min**	**21-25 min**	**26-30 min**	**31-35 min**	**36-40 min**
Vehicle Control	74.83 ± 3.81	3.78 ± 1.37	2.31 ± 1.16	16.38 ± 2.35	39.54 ± 4.76	58.04 ± 7.06	52.58 ± 4.67	29.41 ± 4.16
Syringaldehyde 10 mg/kg	57.95 ± 4.14*	2.50 ± 0.71	2.22 ± 1.38	12.91 ± 3.03	32.64 ± 3.12	52.16 ± 3.31	29.76 ± 3.38***	29.84 ± 3.52
Syringaldehyde 30 mg/kg	63.12 ± 4.60	3.64 ± 1.17	0.93 ± 0.93	19.32 ± 3.16	30.79 ± 4.14	28.37 ± 4.47***	24.50 ± 3.76***	17.21 ± 2.98
Syringaldehyde 100 mg/kg	58.73 ± 5.91*	3.91 ± 0.96	1.60 ± 1.19	20.13 ± 5.30	20.18 ± 5.90**	18.14 ± 2.89***	9.29 ± 1.68***	6.31 ± 1.45***
Naproxen 150 mg/kg	46.49 ± 6.50***	1.32 ± 1.32	0.00 ± 0.00	9.51 ± 1.94	10.30 ± 3.74***	13.68 ± 5.35***	21.86 ± 7.72***	10.57 ± 3.94**

***P<0.001, **P<0.01, *P<0.05 vs Vehicle Control, Two-way ANOVA followed by Bonferroni post-test

Table 3. Effect of Syringaldehyde on Carrageenan induced inflammatory pain in rats

Treatment	Paw withdrawal latency (sec, Mean ± SEM)			
	Baseline	**1 h**	**3 h**	**6 h**
Vehicle Control	10.55 ± 0.47	7.97 ± 0.73	7.06 ± 0.53	7.12 ± 0.61
Syringaldehyde 5 mg/kg	10.50 ± 0.42	8.59 ± 0.81	8.73 ± 0.35	7.48 ± 0.58
Syringaldehyde 15 mg/kg	10.39 ± 0.35	9.78 ± 1.06	11.13 ± 0.86**	9.68 ± 1.77
Syringaldehyde 50 mg/kg	10.37 ± 0.35	10.90 ± 1.46	14.27 ± 1.54***	9.19 ± 0.97

Naproxen 20 mg/kg	10.34 ± 0.35	12.40 ± 0.53**	15.67 ± 0.30***	10.17 ± 1.27
***P<0.001, **P<0.01 vs Vehicle Control, Two-way ANOVA followed by Bonferroni post-test				

Table 4. Effect of Syringaldehyde on CFA induced inflammatory pain in rats

Group	**Paw withdrawal threshold (g, Mean ± SEM)**		
	Pre-CFA Baseline	**Post-CFA Baseline**	**1 h**
Vehicle Control	12.13 ± 0.40	1.70 ± 0.40	1.55 ± 0.39
Syringaldehyde 5 mg/kg	11.20 ± 0.16	1.74 ± 0.39	1.91 ± 0.57
Syringaldehyde 15 mg/kg	12.17 ± 0.45	1.69 ± 0.37	4.02 ± 0.58**
Syringaldehyde 50 mg/kg	11.62 ± 0.40	1.70 ± 0.36	8.07 ± 0.73***
Naproxen 20 mg/kg	11.01 ± 0.35	1.68 ± 0.36	9.17 ± 0.79***
***P<0.001, **P<0.01 vs Vehicle Control, Two-way ANOVA followed by Bonferroni post-test			

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