The Pharma Innovation Journal 2022; SP-11(3): 810-817

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(3): 810-817

www.thepharmajournal.com
Received: 17-01-2022
Accepted: 20-02-2022

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Experimental sub-acute exposure to sodium (Meta) arsenite interferes with pharmacodynamic of meloxicam in Wistar rats

Prashantkumar Waghe, Prakash Nadoor, Vijaykumar M, Sunilchandra U, Rashmi Rajashekaraiah and Pavithra BH

Abstract
The present study aimed to evaluate the modulatory role of sub-acute exposure to sodium (meta) arsenite on pharmacodynamic, *viz.*, antinociceptive, anti-inflammatory, and antipyretic responses mediated by meloxicam in Wistar rats. Rats were divided randomly into 5 groups (n=6). Control rats were given distilled water (Group I). Group II was maintained as Group I but was given meloxicam (2 mg/kg b. wt.) on the 29th day. Groups III, IV, and V rats were given arsenic through drinking water at 0.5, 5.0, and 50 ppm, respectively, for 28 days and meloxicam (2 mg/kg b. wt.) was administered the next day (29th day). For assessment of antipyretic effects, one more additional group (Group VI) was taken and given LPS @ 1.8 mg/kg b. wt. for induction of pyrexia (LPS control). Pain and inflammation were carried out by using formalin-induced nociception and carrageenan-induced inflammatory model(s), respectively by using a standard protocol. The study revealed that experimental rats exposed to higher doses of arsenic showed inhibition of meloxicam-mediated antinociceptive, anti-inflammatory, and antipyretic responses. Further, meloxicam inhibited the arsenic-induced elevation of interleukin-1β, interleukin -6, tumor necrosis factor-α, and COX_2 mediated prostaglandin E_2 in the hind paw muscle. The results intimate that the therapeutic activity of meloxicam unaltered by the presence of arsenic in drinking water within a defined limit, but can interfere the pharmacodynamic when its concentration when relatively high (50ppm). Thus, the observation made has clinical relevance in situations where man and animals are exposed to arsenite epidemic- geographical locations.

Keywords: arsenic, sub-acute exposure, meloxicam, pharmacodynamics, Wistar rats

Introduction
Arsenic, a widely distributed metalloid in the environment. Arsenic poses severe, chronic, and epidemic effects on human, plant, and animal health in South-East Asia [1]. Arsenic contamination in water bodies causes various serious health problems *viz.* skin cancers, internal cancers (bladder, kidney, and lung), diseases of the blood vessels of the legs and feet, possibly diabetes, reproductive disorders, peripheral vascular disease, hypertension, ischemic heart disease, and carotid atherosclerosis etc., due to which World Health Organization guidelines and US Environment Protection Agency (USEPA) set it is the maximum permissible limit of 0.01 ppm through drinking water [2, 3]. Arsenic, a ubiquitous element, is ranked first among toxic agents [4]. Around 150 million people are affected by arsenic toxicity in more than 70 countries [5]. In India, groundwater contamination of arsenic is affecting over 70 million people [6]. There are about 16 affected habitations in the Karnataka State [7] and the groundwater levels of arsenic in Hutti village (Northern part of Karnataka State) were found to 0.09 ppm, which is much higher than the WHO standard [8]. In certain areas in the Indian sub-continent, the maximum arsenic concentration in groundwater was found to around 3.7 ppm to 4.7 ppm [9]. In West Bengal (India), people were exposed to arsenic-contaminated water even in the range of 0.05-14.2 ppm [10].

Subacute arsenic exposure releases pro-inflammatory cytokines tumor necrosis factor- α (TNF- α) and interleukin -1β (IL-1β), which leads to induction of cyclooxygenase (COX) and consequent release of prostaglandin-E_2 (PGE_2) [11]. It has been reported that mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) pathways involved in the arsenic-induced immune-inflammatory process [12].In another study, Liu *et al.* [13] reported that exposure of arsenic to rats produces pro-inflammatory cytokines, which are important mediators of the inflammatory response and are involved with many early systemic inflammation events. Further, chronic low-level arsenic exposure (0.011-0.050 ppm) evokes

chronic inflammation and that, in turn, may lead to susceptibility towards pathogenic infections or in long run may even contribute towards chronic inflammatory diseases including cancer [14].

In India, meloxicam - a nonsteroidal anti-inflammatory drug (NSAID) prescribed in the therapeutic management of pain and inflammation in animals. It blocks COX, the enzyme responsible for converting arachidonic acid into prostaglandin H_2-the first step in the synthesis of prostaglandins (PG), which are mediators of inflammation [15]. Meloxicam was found to inhibit preferentially COX_2 over COX_1 [16]. It has shown increased anti-inflammatory and anti-arthritic activity after oral administration[17]. Meloxicam acts as an analgesic by inhibiting the production of PG and by decreasing sensitization of pain receptors to noxious stimuli [18]. The cytokines (*viz.*, IL-1, IL-6, TNF-α,) and interferons increase hypothalamic PGE_2 synthesis, which in turn, increases cyclic adenosine monophosphate (cAMP) and triggers the hypothalamus to elevate body temperature. Meloxicam suppresses this effect by inhibiting PGE_2 synthesis [17].

A survey of the literature reveals that populations/animals in the arsenic endemic areas of the affected countries are also simultaneously consuming or being administered meloxicam for medical reasons. Further, assessment of the potential modulatory role of arsenic on the therapeutic efficacy of therapeutic agents is also required to ensure the effectiveness of the prescribed medicines in the arsenic-exposed subjects. Arsenic was shown to over express COX_2, up-regulate inflammatory cytokines, and exacerbate inflammatory and painful responses [19]. Therefore we assumed that arsenic exposure can influence pharmacodynamics of NSAIDs. To test this hypothesis, we evaluated the effect of arsenic exposure (subacute) on pro-inflammatory mediators linked to the efficacy of meloxicam and assessed the pharmacodynamics of meloxicam in experimental rats exposed to arsenic

Materials and Methods

Male Wistar rats (200-230 g) obtained from the Laboratory Animal House of the Veterinary College, Shivamogga were employed for the study. The animals were maintained under standard practices as per the guidelines of the committee for supervision and control of animal experiments (CPCSEA, New Delhi). The animal experiments were imitated after necessary approvals from the Institutional Animal Ethics Committee (IAEC Approval No.VCS/IAEC/012/ 2017-18 Dated: 10.06.2017). Before the experiment, all the animals were kept in laboratory conditions for 7 days or more for acclimatization.

Rats were exposed to arsenic as sodium arsenite through drinking water for 28 days. A single dose of meloxicam (2 mg/kg b. wt.) was administered by oral gavage on the 29th day. The exact time of meloxicam administration depended on the type of test. Rats were divided randomly into 5 groups (n=6). Control rats were given distilled water (Group I). Group II was maintained as Group I but was given meloxicam on the 29th day. Groups III, IV, and V rats were given arsenic through drinking water at 0.5, 5.0, and 50 ppm, respectively, for 28 days and meloxicam was administered the next day (29th day). For assessment of antipyretic effects, one more additional group (Group VI) was taken and given LPS @ 1.8 mg/kg b. wt. for induction of pyrexia (LPS control). Pain and inflammation were carried out by using formalin-induced nociception and carrageenan-induced inflammatory model(s), respectively by using a standard protocol.

At the end of the study, animals were euthanized by anesthetic overdose. Muscles from the hind paws of the rats used for formalin-induced nociception and carrageenan-induced inflammation were collected and rinsed with saline. Immediately after the tissue collection, the tissue was stored at - 20◦ C until further analysis.

Assessment of antipyretic effect in lipopolysaccharide (LPS)-induced pyrexia

LPS-induced pyrexia was assessed by the method of Santos and Rao [20]. LPS was dissolved in pyrogen-free normal sterile saline (1.8 mg/ml). The normal rectal temperature was recorded with a clinical thermometer before LPS administration. Then the rats were injected intraperitoneally either saline (control group) or LPS (1.8 mg/kg) and the rectal temperature was measured at different hours. The time of LPS administration was taken as 0 h. The rectal temperature rose to the maximum during 6-7 h. The LPS-treated rats were given orally either vehicle or meloxicam at 6 h to examine whether temperature rise could be reversed. The temperature was further recorded after 3 h of meloxicam treatment.

Assessment of anti-inflammatory in carrageenan-induced hind-paw edema

This test was conducted as per the method described by Winter *et al*., [21]. The paw volume was measured using a calibrated Plethysmometer. The phlogistic agent carrageenan was prepared as 1% suspension in normal sterile saline and injected subcutaneously (0.1 ml) into the plantar aponeurosis of the right hind paw. To evaluate the anti-inflammatory activity, meloxicam was administered orally 1 h before the injection of carrageenan. Normal paw volume was recorded (0 h volume) before meloxicam administration. The edema volume of the injected paw was measured at 3 h following carrageenan injection. The inflammatory effect of carrageenan was assessed in terms of the difference in the paw volume at '0' h from the volume at 3 h following carrageenan injection. From the mean edema volume, the percentage of inhibition of edema was calculated between treated and control groups.

Assessment of analgesic activity in formalin-induced nociception

To examine the effects of arsenic on formalin-induced nociceptive behavior, formalin was diluted to 5% from a stock solution of 100% (formaldehyde solution, 37%, v/v) and injected (50 µl) subcutaneously into the left hind paw. Immediately after the formalin injection, the rats were placed in a glass funnel and observed continuously. Nociceptive behavior was evaluated as the number of flinches, i.e., rapid and brief withdrawal or flexing of the injected paw (Dubbuisson and Dennis, [22]. The typical biphasic pattern of formalin-induced flinching was identified as an initial acute phase (0-15 min, I phase) followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (30-60 min, II phase) [23]. The nociceptive response was expressed as the total number of flinches in each phase. Meloxicam was administered 1 h before formalin injection.

Enzyme-Linked Immunosorbent Assay (ELISA)

The level of IL-1β, Interleukin-6 (IL-6), TNF-α (RayBiotech Inc., USA), and production of PGE_2 assay (Elabscience® Biotechnology Inc., Wuhan, China) in paw muscle were measured by using ELISA kits as per the manufacturer's standard protocol.

Statistical Analysis

The values obtained from the various experiments were expressed as Mean ± S.E with 'n' equal to the number of animals. Data obtained were statistically subjected to one-way analysis of variance (ANOVA) followed by Duncan's*post hoc* multiple comparison test using SPSS statistic software (IBM® SPSS® statistic software, Version 20.0.0, 2011, Armonk, NY, USA). The difference was considered significant at $p<0.05$. Graphical presentation of the data was carried out by using the Graph Pad Prism software program (GraphPad® software Inc., Version 7.0; San Digo, CA, USA).

Results

The influence of arsenic preexposure on the anti-inflammatory effect of meloxicam in carrageenan-induced inflammation in rat paw has been presented in Fig. 1. The edema volume control and meloxicam-treated rats were 1.39±0.11 and 0.81±0.05 (41.72%) respectively. Meloxicam significantly ($p < 0.05$) decreased the control volume by 28.05% (1.00±0.06) with 0.5 ppm and by 23.74% (1.06±0.05) with 5 ppm of arsenic preexposure. But arsenic preexposure in 50 ppm significantly increased the edema volume to 1.38±0.07 compared to the meloxicam treated group (0.81±0.05). The edema volume in this group did not significantly reduce by the meloxicam.

Fig. 2 presents the influence of arsenic pre-exposure on the analgesic effect of meloxicam in formalin-induced nociception in the rat paw. The total number of flinches in the control group of rats during the early and late phases was 32.67±1.45 and 41.33±2.18 respectively. In 0.5 ppm and 5 ppm arsenic exposure groups meloxicam significantly ($p < 0.05$) decreased the number of flinches by 26.54% (24.00±1.16) and 27.55% (23.67±1.86) in the early phase (Phase I), and by 45.97% (22.33±1.45) and 45.15% (22.67±2.40) in the late phase (phase II). However, at a higher concentration of arsenic (50 ppm) meloxicam did not prevent the nociception as indicated by the significant increase in flinches. The *percent* increase in flinches during the early and late phase were respectively 04.10% (31.33±2.19) and 06.44% (38.67±1.20) as compared to control.

The influence of arsenic preexposure on the anti-pyretic effect of meloxicam in LPS-induced pyrexia in the rat at different time intervals in experimental rats. Arsenic itself did not increase the body temperature after 28 days of exposure. LPS significantly increased the body temperature compared to the control group of rats after 6 hours of its administration. LPS induced pyrexia was significantly reduced by the meloxicam in 0.5 and 5 ppm arsenic exposure groups at 7.5 hours and 9 hours. The antipyretic effect of meloxicam is significantly altered when arsenic pre-exposure (50 ppm) compared to the control and LPS treated group of rats (Table 1)

Fig. 3 presents the influence of arsenic preexposure on meloxicam-mediated alteration in IL-1β (pg/mg protein) level in carrageenan-induced inflammation in the rat paw. In the control rats, its level was 122.11±8.35. It was significantly reduced with meloxicam to 56.18±5.78 (53.99%). In the 0.5 ppm and 5 ppm arsenic-exposed rats, meloxicam was significantly decreased its level to 68.28±5.00 (49.00%) and 79.70±4.69 (34.73%), respectively. However, at a higher concentration of arsenic (50 ppm) meloxicam did not reduce its level 118.24±3.91 (03.17%) and it was almost similar to the control group.

Fig. 4 shows the influence of arsenic preexposure on meloxicam-mediated alteration in IL-1β level (pg/mg protein) in formalin-induced nociception in the rat paw. In the control rats, its level was 130.44±8.52. Meloxicam significantly decreased the production of IL-1β to 67.18±3.73 (48.50%). In the 0.5 ppm and 5 ppm arsenic-exposed rats, IL-1β level was significantly decreased by meloxicam to 61.62±5.08 (52.76%) and 97.53±4.98 (25.23%) respectively. And it was 50 ppm of the arsenic-exposed rats its level was comparable to control 124.91±7.98 (04.24%).

In carrageenan-induced inflammation, the level of IL-6 (pg/mg protein) was 340.95±19.07 in the control rats. Meloxicam treatment causes significant alteration in its level to 211.18±05.55 (38.06%). In the 0.5 ppm and 5 ppm arsenic-exposed rats, IL -6 levels were significantly reduced by meloxicam to 201.95±08.13 (40.77%) and 268.03±18.31 (21.39%) respectively. Meloxicam treatment did not reduce the IL-6 levels (322.41±12.24; 05.44%) in the 50 ppm arsenic-exposed rats (Fig. 5).

Fig. 6 presents the arsenic preexposure on meloxicam-mediated alteration in IL-6 level in formalin-induced nociception in the rat paw. In the control rats, IL -6 levels (pg/mg protein) were 347.61±23.67. In the 0.5 ppm and 5 ppm arsenic-exposed rats, meloxicam brought back the IL-6 level to 230.28±11.77 (33.76%) and 284.70±12.08 (18.10%), respectively to the meloxicam group 234.52±09.37 (32.53%). However, 50 ppm exposure to arsenic significantly inhibited the effect of meloxicam 325.74±05.71 (06.29%).

In carrageenan-induced inflammation, the control level of TNF-α production in rat paw muscle was 63.18±1.96 pg/mg protein (Fig. 7). Meloxicam treatment significantly decreased these production levels by 38.81% to 38.66±2.27 pg/mg protein. The anti-inflammatory effect of meloxicam observed with 0.5 ppm and 5 ppm of arsenic was significant and their level in the respective group were 41.71±1.40 (33.98%) and 47.35±2.97 (25.06%). 50 ppm of arsenic preexposure inhibited the effect of meloxicam to 59.28±1.78 (06.17%).

Fig. 8 summarizes the TNF-α production (pg/mg protein) in formalin-induced nociception in the rat paw. The levels of its production in the control (68.02±4.68) and the meloxicam (26.71±1.24) rats were statistically comparable. Rats treated with 0.5 ppm and 5 ppm of arsenic exhibited a significant ($p<0.05$) decrease in its production by meloxicam and the respective values were 28.49±2.23 (58.12%) and 52.85±3.08 (22.30%). The effect of meloxicam was significantly inhibited in the 50 ppm arsenic-exposed rats to 64.28±5.04 (05.50%).

Fig. 9, shows the effects of carrageenan-induced inflammation on the PGE_2 level in the paw muscle. In carrageenan-induced nociception, the level was 17.91±0.65 pg/mg protein in the control rats. Meloxicam significantly decreased these to 10.75±0.83 (39.98%). Lower concentration of arsenic at 0.5 ppm and 5 ppm meloxicam significantly modify its level to 12.67±0.47 (29.26%) and 14.03±0.61 (21.67%) respectively. At 50 ppm concentration, arsenic caused significant and comparable inhibition of meloxicam-mediated effect 16.53±0.80 (07.71%).

The alterations in PGE_2 levels in paw muscle of rats used for formalin-induced nociception Fig. 10. PGE_2 level in the respective control rats was 19.07±0.63 (pg/mg protein). Meloxicam significantly decreased the production of PGE_2 by 29.58% to 13.43±0.64. This effect of meloxicam was significantly reduced by 13.12±0.23 (31.20%) and 16.10±0.48 (15.57%) with 0.5 ppm and 5 ppm concentration of arsenic preexposed groups, respectively. However, the higher concentrations of arsenic (50 ppm) significantly and comparably to control inhibited the effect of meloxicam by 18.61±1.34 (02.41%).

Table 1: Influence of arsenic preexposure on anti-pyretic effect of meloxicam in lipopolysaccharide (LPS)-induced pyrexia in experimental rats

Treatment	Dose	Rectal Temperature (^{0}F)						
		0 hrs	1.5 hrs	3.0 hrs	4.5 hrs	6.0 hrs	7.5 hrs	9.0 hrs
Control	-	96.23±0.41^a	96.33±0.43^a	96.50±0.46^a	96.20±0.10^a	96.30±0.45^a	96.57±0.65^a	96.67±0.30^a
LPS	1.5 mg/kg IP	96.27±0.23^a	96.27±0.35^a	96.00±0.29^a	95.70±0.50^a	99.67±0.39^b	97.40±0.47^b	97.57±0.64^b
LPS+ Meloxicam	1.5 mg/kg IP + 1.5 mg/kg PO	96.17±0.39^a	96.33±0.47^a	96.70±0.23^a	96.30±0.58^a	96.40±0.21^a	95.93±0.35^a	95.63±0.38^a
Arsenic + Meloxicam	0.5 ppm + 1.5 mg/kg	96.73±0.28^a	96.63±0.26^a	96.23±0.07^a	96.47±0.60^a	99.70±0.35^b	96.90±0.31^a	96.67±0.66^a
Arsenic + Meloxicam	5 ppm + 1.5 mg/kg	96.73±0.28^a	96.80±0.26^a	96.43±0.38^a	96.50±0.72^a	100.40±0.53^b	96.83±0.24^a	96.67±0.70^a
Arsenic + Meloxicam	50 ppm + 1.5 mg/kg	96.53±0.22^a	96.20±0.32^a	96.90±0.25^a	96.47±0.60^a	101.00±0.61^b	101.30±0.76^b	101.73±0.22^b

Values (mean ± SE; n = 6) bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparison post-hoc test.

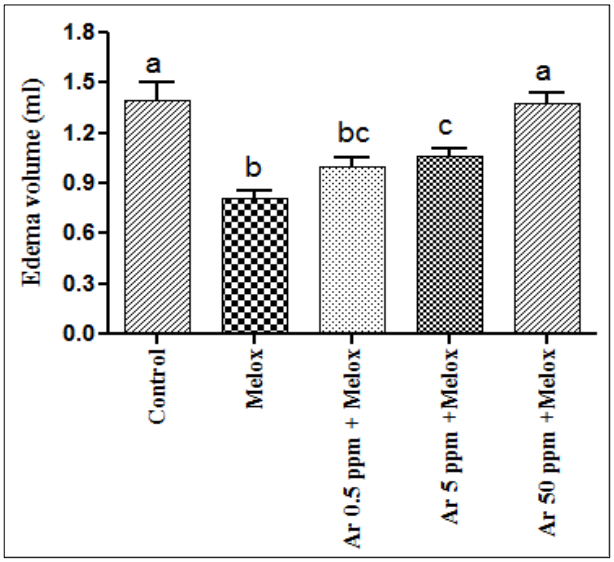

Fig. 1: Influence of arsenic preexposure on the anti-inflammatory effect of meloxicam in carrageenan-induced inflammation in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing no common superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test.

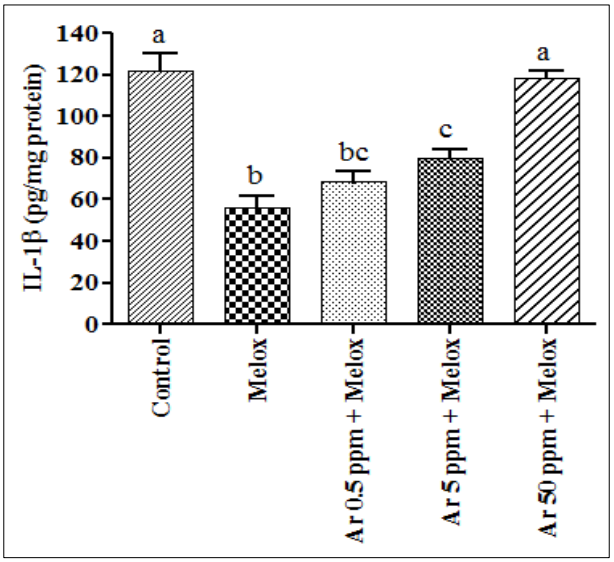

Fig. 3: Influence of arsenic preexposure on meloxicam-mediated alteration in interleukin-1β (IL-1β) level in carrageenan-induced inflammation in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test.

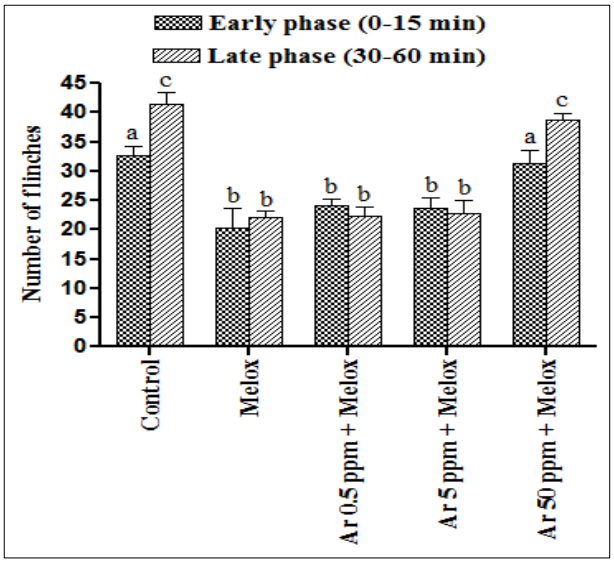

Fig 2: Influence of arsenic preexposure on the analgesic effect of meloxicam in formalin-induced nociception in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test

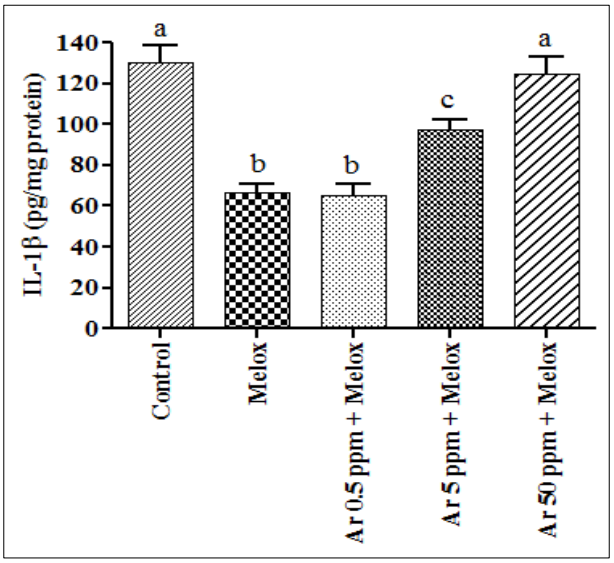

Fig 4: Influence of arsenic preexposure on meloxicam-mediated alteration in interleukin-1β (IL-1β) level in formalin-induced nociception in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test.

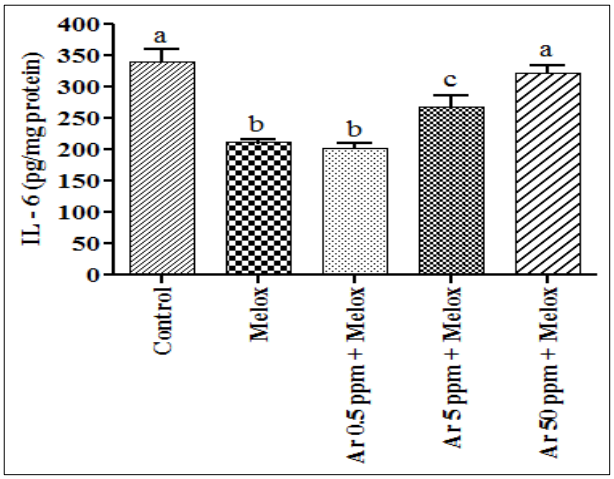

Fig 5: Influence of arsenic preexposure on meloxicam-mediated alteration in interleukin-6 (IL-6) level in carrageenan-induced inflammation in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test.

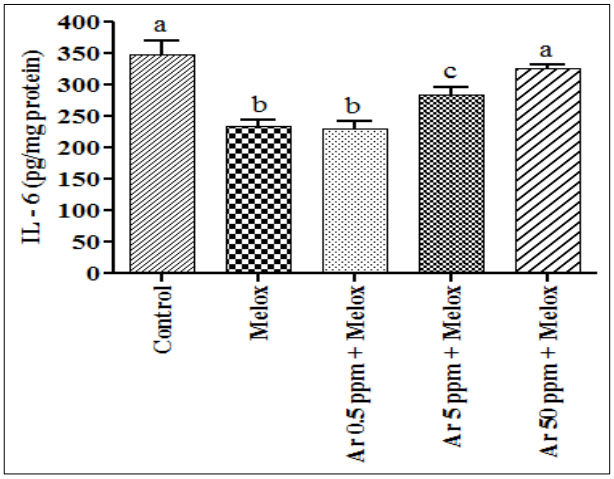

Fig 6: Influence of arsenic preexposure on meloxicam-mediated alteration in interleukin-6 (IL-6) level in formalin-induced nociception in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test.

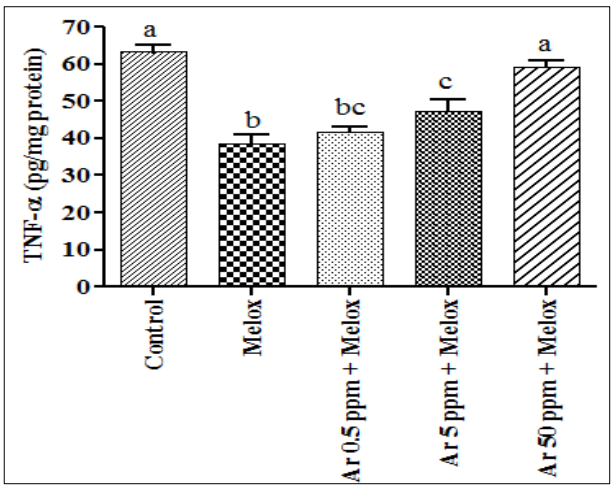

Fig 7: Influence of arsenic preexposure on meloxicam-mediated alteration in tumor necrosis factor-alpha (TNF-α) level in carrageenan-induced inflammation in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test.

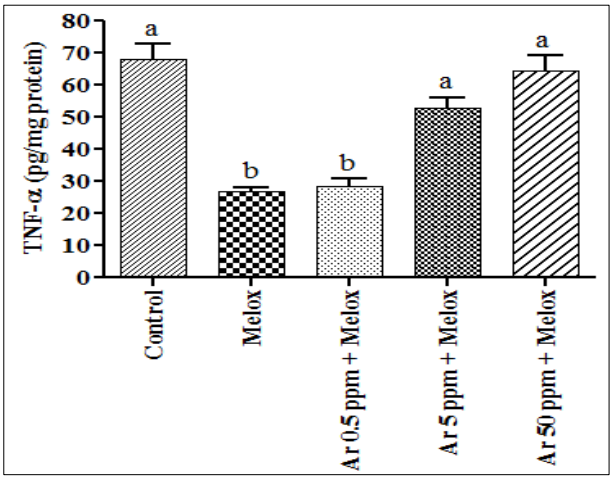

Fig 8: Influence of arsenic preexposure on meloxicam-mediated alteration in tumor necrosis factor-alpha (TNF-α) level in formalin-induced nociception in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test.

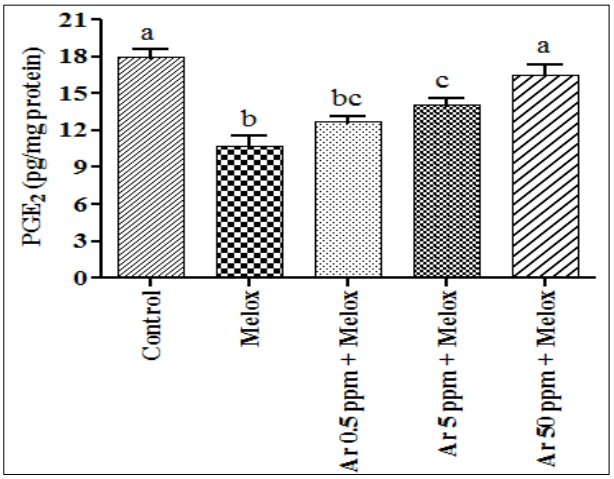

Fig 9: Influence of arsenic preexposure on meloxicam-mediated alteration in prostaglandin E_2 (PGE_2) level in carrageenan-induced inflammation in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test.

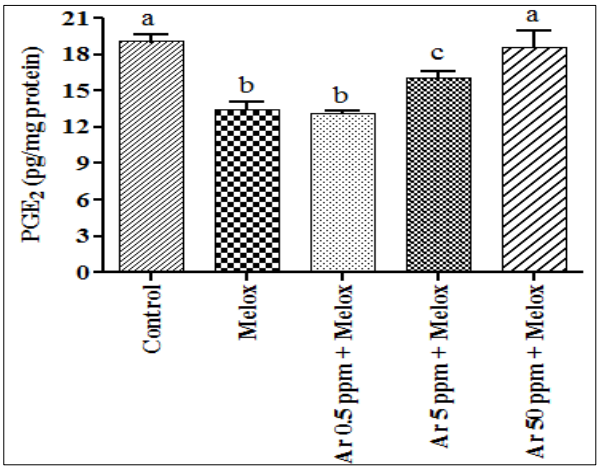

Fig 10: Influence of arsenic preexposure on meloxicam-mediated alteration in prostaglandin E_2 (PGE_2) level in formalin-induced nociception in the rat paw. Each bar represents mean ± standard error (n = 6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparisons *post-hoc* test.

Discussion

In the current study subacute sodium (meta) arsenite exposure through drinking water reduces antipyretic, anti-inflammatory, and analgesic effects of meloxicam. Arsenic at 0.5 ppm and 5 ppm did not significantly modify the effect of meloxicam. At 50 ppm concentrations, arsenic caused significant and comparable inhibition of meloxicam-mediated effects. In certain areas in the Indian subcontinent, the maximum arsenic concentration in ground water was found to be around 3.7 ppm to 4.7 ppm [9]. In West Bengal (India), people were exposed to arsenic-contaminated water even in the range of 0.05-14.2 ppm [10]. Therefore, prolonged exposure to such environmental concentrations of arsenic in humans/animals could decrease the therapeutic efficacy of meloxicam.

Inflammation is a complex process that occurs due to the participation of a large number of vasoactive, chemotactic, and proliferative factors at different stages. Prostaglandins, leukotrienes, platelet-activating factors, cytokines are released by the host of mechanical, chemical, thermal, and bacterial insults which contribute to the genesis of inflammation [24]. The carrageenan-induced paw edema is a distinct model of acute inflammation that a variety of inflammatory mediators involves in its enlargement and it has been used to evaluate the anti-edematous effect of meloxicam. This model is a short-term process that is characterized by typical signs of inflammation, such as swelling, heat, redness, and pain [21]. Cytokines are the most important molecules in inflammatory responses and their regulation during inflammatory activities [25]. Intraplantarinjectionofcarrageenanledtotime-dependent development of peripheral inflammation, which resulted in a significant increaseinthelevelsofTNF-α andIL-1β, nitricoxide(NO)andPGE2 andalso*i*NOSandCOX_2proteinexpressioninflamed paw [26]. Acute [27], sub acute[11] and chronic [14] exposure to arsenic induce inflammation by shifting the levels of the pro-inflammatory cytokines and PGE_2. In the present study, we showed that meloxicam produced anti-inflammatory effects by reducing the level of pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and COX2 mediated PGE2 in carrageenan-induced rat paw edema in 0.5 ppm and 5 ppm arsenic preexposure rats. However, the higher concentrations of arsenic (50 ppm) significantly and comparably to control inhibited the effect of meloxicam. Similar to our findings, Dudhgaonkar*et al*., [28] concluded that administration of meloxicam produces anti-inflammatory effects in the carrageenan-induced inflammation in experimental rats.

The formalin test involves a biphasic response with an early and a late phase representing neurogenic and inflammatory pain [29]. PGs play an important role in nociceptive information transmission in the peripheral and central nervous systems [30]. COX_1 being a constitutive enzyme considered necessary for normal homeostasis, while COX_2 is induced by cytokines such as IL-1. Inflammatory conditions can catalyze the conversion of arachidonic acid to PGs [24]. In the present study, meloxicam decreased the number of flinches during the early (0-15 min) phase I and late (30-60 min) phase II in arsenic pre-exposure rats. Aguirre-Banuelos *et al*., [31] reported that acute or subchronic inorganic arsenic exposure enhances pain perception and exacerbates the pathological state of inflammatory diseases. Further, Ahmad *et al*., [11] suggested subacute arsenic exposure through drinking water increases the COX activity and the local release of PGE2 assisted in the mechanism of exacerbation of pain and inflammation. In another study, demonstrated that arsenite exposure induces COX_2 expression *via* the NFAT-dependent pathway in human bronchial epithelial *Beas*-2B cells [32] and mouse epidermal Cl41 cells [33] increasing in prostaglandin concentrations [31]. These prostaglandins (mainly PGE2 and PGI2) sensitize the nociceptive afferent nerve terminals to mediators like bradykinin, cytokines (IL-1β, IL-8, TNF-α), and some other analgesic agents to produce intense pain [34]. In the present study, meloxicam inhibits the COX pathway leading to decreased production of PGE_2 and desensitized the nociceptive receptors to cytokines IL-1β, IL-8, TNF-α in rats pre-exposed to arsenic.

We also investigated the antipyretic effect of meloxicam on the bacterial LPS-induced fever model in arsenic pre-exposure rats. It is reported that LPS-induced fever in rats is generally polyphasic and special mechanisms underlie different febrile phases [35]. The mechanism of fever initiation and maintenance is a complex process. It is generally believed that the release of LPS from Gram -negative bacterial cell walls during most infections can cause a fever by stimulating peripheral macrophages to synthesize and release pyrogenic cytokines such as IL-1β, IL-6, TNF-α that induce PGE2 production, in and near the hypothalamic area. The PGE2 via an increase in cyclic AMP triggers the hypothalamus to elevate the set point at which body temperature is regulated resulting in fever [36]. Cytokines either directly or through the induction of PGs can also stimulate vasomotor centers in central nervous system resulting in sympathetic nerve stimulation, peripheral vasoconstriction, decrease in heat dissipation, and fever [24]. In the present study, arsenic itself did not increase the body temperature after 28 days of exposure but, LPS significantly increased the fever after 6 hours of its administration. Meloxicam repressed LPS induced pyrexia in 0.5 and 5 ppm arsenic exposure groups at 7.5 hours and 9 hours. However, meloxicam did not show an antipyretic effect in 50 ppm arsenic pre-exposure rats. But at higher concentrations, the arsenic-mediated inhibitory effect of meloxicam could be linked to pro-inflammatory cytokines and consequent release of PGE_2 that induces more fever. Meloxicam has weak effects against pyrogen fever and it is not an alternative to conventional antipyretic drugs [16].

To conclude, that experimental sub-acute exposure to arsenic through drinking water aggravate pyrexia, inflammation, and pain at environment relevant concentration and caninterfere the pharmacodynamic properties of meloxicam at a higher level of arsenite (As^{+3}) exposure. Thus, the observation made has clinical relevance in situations where animals are exposed toarsenic epidemic geographical locations.

Acknowledgements

The authors are thankful to the Dean, Veterinary College, Shivamogga, Karnataka (India) for providing necessary facilities to conduct the study.

Declaration of Conflicting Interests

The authors have no conflict of interest to declare.

Funding

The authors are grateful to Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU), Nandinagar, Bidar, Karnataka State, India, Pin code: 585 226 (India), for providing financial support in conducting the study. (Ref: DR/KVAFSU/SRP Pre and Para ClinSc-20/2016-17 dated: 31.07.2016).

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