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Anti-nociceptive and Anti-inflammatory Effect of *Monotheca buxifolia* Leaves and Bark Extract: A Comparative Approach

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ABSTRACT

Background: Inflammation plays an important role in various diseases with high prevalence within populations. *Monotheca buxifolia* has been long used in the folk medicine in urinary tract infections.

Objective: The aim of the study was to comparatively evaluate the anti-nociceptive and anti-inflammatory activities of *M. buxifolia* leaves and bark along with their fractions.

Methods: The anti-nociceptive activity of *M. buxifolia* was assessed via acetic acid induce writhing test. The anti-inflammatory activity was determined via formalin induce paw method, while *in vitro* anti-inflammatory and anti-nociceptive activity was carried out through COX-2 enzyme inhibition.

Results: The acetic acid induce anti-nociceptive results of *M. buxifolia* of bark were more significant ($p<0.001$) then leaves crude extract of *M. buxifolia* at 500 mg/kg. The chloroform fraction of both leaves and bark extract also depicts significant reduction 26.5±1.0 and 23.5±0.8 in number of writhes respectively at 500 mg/kg. The anti-nociceptive effect of *M. buxifolia* bark in paw licking was also significant. The paw licking duration with *M. buxifolia* leaves crude extract was 1.9±0.01 min at 500 mg/kg dose ($p<0.001$) in the early phase. The chloroform fraction also showed significant ($p<0.001$) reduction in paw licking duration. The COX-2 inhibitory assay of leaves extract of *M. buxifolia* exhibited 60.0±0.5% inhibition ($p<0.001$) at 1000 µg/ml. The chloroform fraction of *M. buxifolia* leaves at 1000 µg/ml showed 42.80 ±0.3 inhibition. The bark crude extract of *M. buxifolia* inhibited 63.83±0.73 % ($p<0.001$) COX-2 at 1000 µg/ml. The chloroform fraction of *M. buxifolia* bark at 1000 µg/ml showed 43.83 ±0.33 % inhibition. The results were also comparable with standard drug diclofenac sodium.

Conclusion: Based on promising anti-nociceptive and anti-inflammatory results, it is suggested that *M. buxifolia* should be a part of complementary and alternative medicine.

Keywords: *Monotheca buxifolia, anti-nociceptive, anti-inflammatory, COX inhibition.*

INTRODUCTION

Inflammatory responses have key protective role in various pathological conditions that occur in acute and chronic phase. Inflammatory response is characterized by chemotactic movement of leukocytes and phagocytes into inflamed site [1,2]. The inflammatory process has a clear relationship with fatigue that leads to nociception [3]. Pain is an unpleasant sensation which is induced by deleterious and/or potentially damaging noxious stimuli. Multiple aspects including various biological, psychological conditions and socio-cultural attributes have rendered pain perceptions [4,5,6]. Non-steroidal anti-inflammatory drugs (NSAID's) and opioid analgesic are still used in the management of pain and inflammation. Nonetheless, these drugs are effective but have side effects like damage of renal and mucosal membrane. Similarly, respiratory depression, dependency, and tolerance are related with opioids analgesics [7]. Therefor plants have an essential role in the discovery of novel anti-inflammatory and analgesic drugs [8]. *M. buxifolia* from family Sapotaceae fruit has been previously evaluated for potent anti-inflammatory, anti-nociceptive, anti-pyretic by [9], antioxidant by [10]. The fruit of *M. buxifolia* also have renal and hepatoprotective activity [11, 12]. The barks of *M. buxifolia* have neuropharmacological and antidepressant like activity [13], while leaves of *M. buxifolia* antibacterial and anticancer activity has also been previously reported [14].

Even though, the barks and stem of this plant are traditionally used topically in the treatment of inflammation due to bacterial infections particularly in rural areas of Pakistan. However, there is no scientific data reported on the comprehensive in vivo anti-inflammatory activity of barks and stem of *M. buxifolia*. Also, investigation of the faithful pharmacological potential of this plant is highly required given the pervasive use. Therefore, the present study was aimed at to compare and standardize the leaves and barks of *M. buxifolia* for anti-inflammatory and anti-nociceptive purpose.

MATERIALS AND METHODS

The identification of plant and extraction process was carried out as previously explained in our previous study [13]. Fresh leaves and barks of *M. buxifolia* were purchased from local commercial market of Karachi, Pakistan. It was identified by the Pharmacognosist at Center of Plant Conversation, University of Karachi, Pakistan. The final product was a dark brownish sticky mass weighing 7.3 and 11.5 g for leaves and barks respectively.

Ethical approval

Use of animals in this study was in accordance with the National Advisory Committee for Laboratory Animal Research guide for the care and use of Laboratory Animals and under the acquiescence of the Board of Advance Studies and Research, University of Karachi, Pakistan. The study was approved by research review committee of Federal Urdu University of Arts, Science and Technology with the reference number of 00298.

Anti-nociceptive activity

In-vivo analgesic activity was measured in 15 h fasted Swiss mice of either sex (16-25 gm, six animals in each group) by acetic acid- induced writhing and formalin induced inflammatory methods.

Acetic acid-induced writhing test

The analgesic potential of *M. buxifolia* was evaluated by acetic acid test. The animals were divided into five groups (n=6) mice in each group. Control group I was treated with normal saline (0.5 ml/ per animal). Group II received diclofenac sodium 10 mg/kg (standard). Group III, IV and V were treated with plant samples in various concentrations. After the administration of acetic acid, the mice were observed for 5-30 minutes for the contraction movements in the abdomen, twisting trunk, and body elongation [15].

Formalin induced anti-inflammatory activity

M. buxifolia leaves and bark paw edema were investigated for anti-inflammatory activity using formalin induced licking test. The test samples i.e. crude methanolic extracts of leaves and bark and their subsequent fractions were orally administered to animals. Formalin was injected after 30 minutes into sub-planter surface of the hind paw of the mice at a dose of 2.5 % (20 µl) (v/v in distilled water). Group-I negative control received normal saline at a dose of (0.5 ml/ per animal). The standard drug diclofenac sodium was administered to group II, 10 mg/kg. Group III, IV, and V received orally 100, 300, and 500 mg/kg of samples and their fractions. Paw licking by mice was indicated as nociceptive behavior. The total time spent by licking and biting of paw was recorded. The earliest 5 min was regard as neurogenic phase,

while the next 15-30 min is considered as the inflammatory phase of the nociceptive response [16]. The anti-inflammatory response was calculated using paw thickness for 2.5 h.

Cyclooxygenase-2 (COX-2) assay

The cyclooxygenase assay was performed similarly as previously reported by [17]. The solution of COX-2 enzyme was prepared at 300 U/ml. The 10 µl enzymatic solution was activated for 5 min on ice along with cofactor (50 µl) having glutathione 0.9 mM, N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) 0.24 mM and one mM hematin in 0.1 M tris buffer (pH 8). The 60 ml enzyme solution and the test samples of concentration (1000-62.5 µg/ml) were kept at room temperature for 5 min. Afterwards, about 20 µl of arachidonic acid having concentration of (30 mM) was added to initiate the reaction followed by incubation for 5 mints. The absorbance was measured at 570 nm and the COX-2 inhibitory percentage was calculated using celecoxib as positive control.

RESULTS

Analgesic activity

Acetic acid induced writhing test

Various doses 100, 300, and 500 mg/kg, p.o. of *M. buxifolia* leaves and bark extract with their fractions were orally administered to different groups of mice. elicit a significant analgesic activity in acetic acid induce writhing test. The results of *M. buxifolia* of bark were more significant then leaves crude extract of *M. buxifolia* leaves extract at 500 mg/kg (Figure **1b**). The results were dose dependent, like at 100 mg/kg the total number of writhes were 39.8±1.8 and 37.6±1.2 of leaves and bark CME respectively (Figure **1a** and 1b). The results were significantly less i.e. 22.0±0.62 and 20.4±0.8 total writhes at 500 mg/kg. The Chloroform fraction CHF of both leaves and bark extract also depicts significantly reduced 26.5±1.0 and 23.5±0.8 number of writhes respectively at 500 mg/kg as compared with control and other fractions as shown in (Figure **1a** and **1b**).

Formalin induce anti-inflammatory activity

The paw licking duration at different concentrations i.e. 100, 300 and 500 mg/kg was significantly reduced upon administration of *M. buxifolia* crude leaves and bark extract (Figure **2** and **3**). With administration of leaves crude extract, the paw licking duration was 1.9±0.01 mint at ($p<0.001$) at 500 mg/kg dose in the early phase. In late phase the paw licking 3.1±0.04 ($p<0.01$) (Figure **2**). The chloroform fraction also showed significant ($p<0.001$) reduction in paw licking duration as shown in (Figure **2d**). The *M. buxifolia* bark crude extract showed 1.6±0.01 mint ($p<0.001$) duration of licking in early phase at 500 mg/kg dose. In late phase the duration of licking was 2.9±0.04 mint ($p<0.01$) (Figure **3**). The bark chloroform fraction also exhibited a dose dependent paw licking inhibition. In early phase the paw licking duration was 2.1±0.0 mints ($p<0.001$), while in late phase the paw licking duration was 3.1±0.02 mints ($p<0.01$) to 2.3±0.0 minutes as shown in (Figure **3d**). The results of *M. buxifolia* crude leaves and bark extract and its chloroform fractions were comparable with standard drug diclofenac sodium.

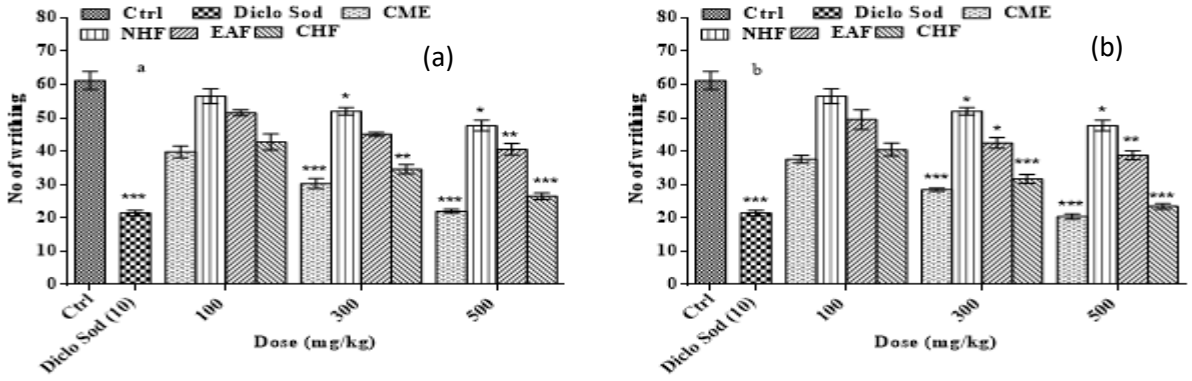

Figure 1. Antinociceptive effect of (a) *M. buxifolia* crude leaves and (b) *M. buxifolia* crude bark and their fraction on acetic acid-induced writhing response. Values are expressed as mean ± S.D. *n*=6 in each group. Statistical significance: *p<0.05; **p<0.01; ***p<0.001.

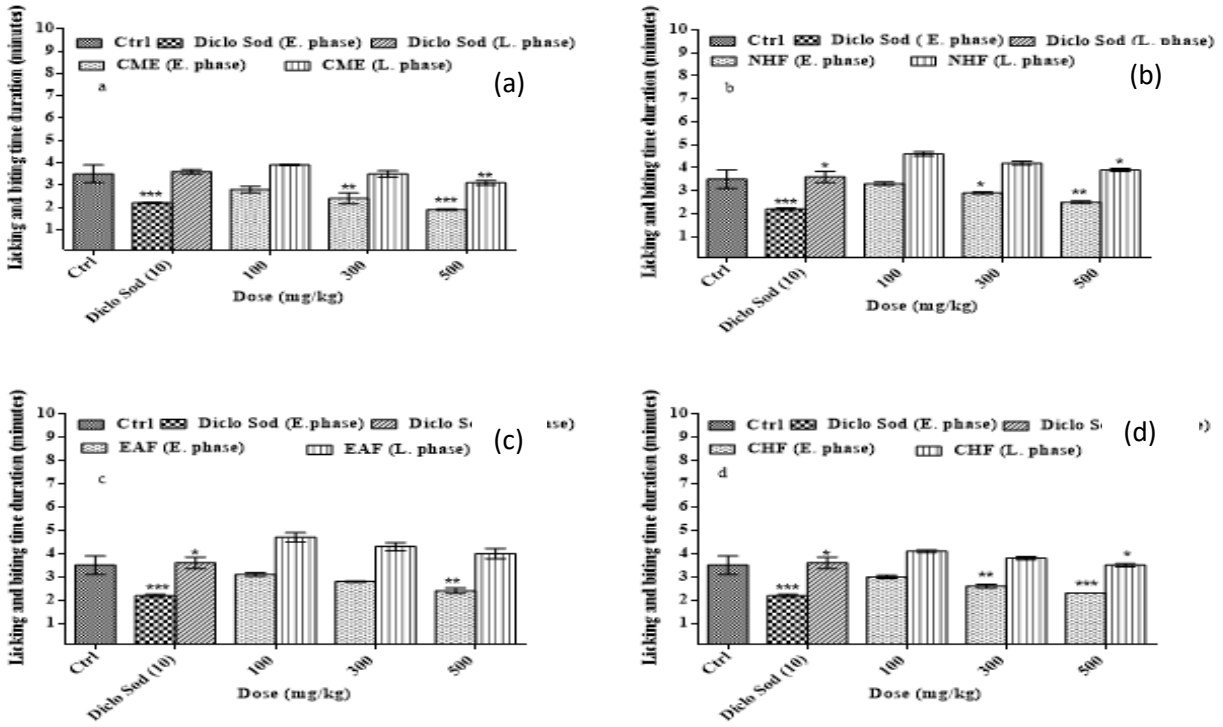

Figure 2. Antinociceptive activity of *M. buxifolia* leaves (a) CME= crude methanolic extract (b) NHF= *n*-hexane fraction (c) EAF= ethyl acetate fraction (d) CHF= chloroform fraction, in formalin induced paw licking and biting assay. Values expressed as mean ±SEM. *p<0.05, **p<0.01, ***p<0.001. *n*=6.

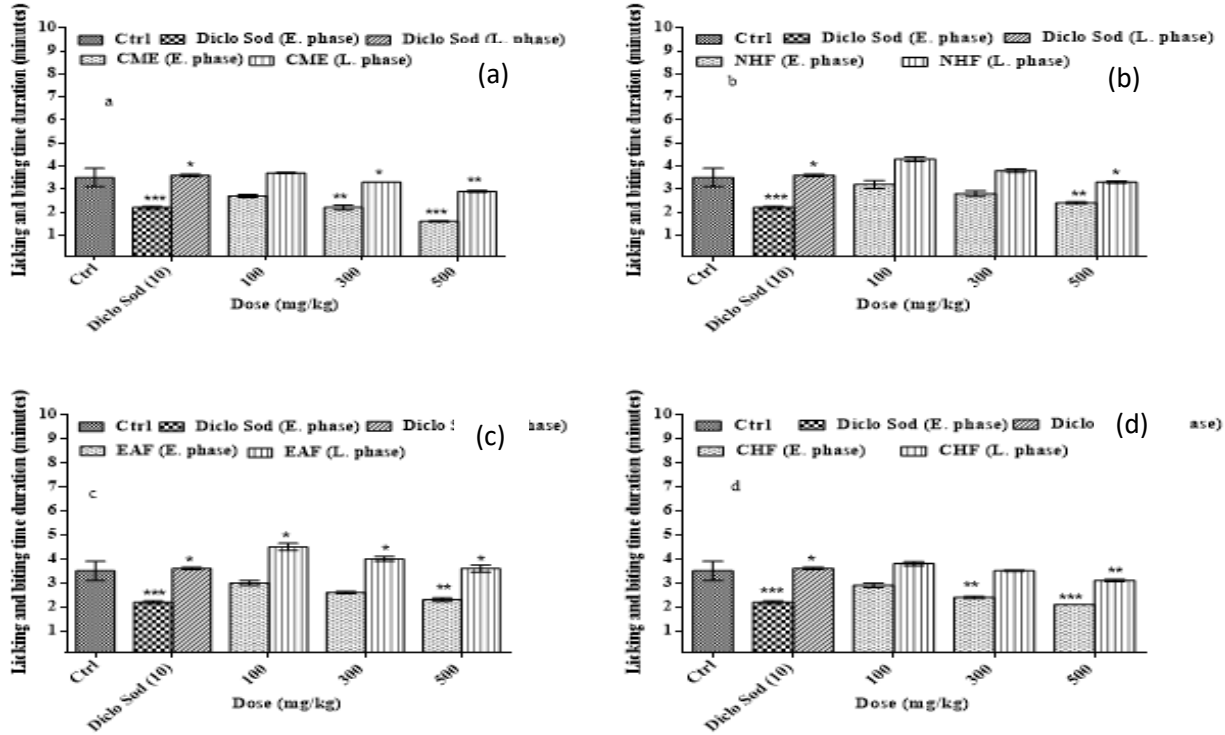

Figure 3. Antinociceptive activity of *M. buxifolia* bark (a) CME= crude methanolic extract (b) NHF= *n*-hexane fraction (c) EAF= ethyl acetate fraction (d) CHF= chloroform fraction, in formalin induced paw licking and biting assay. Values expressed as mean ±SEM. *p<0.05, **p<0.01, **p<0.001. *n*=6.

The anti-inflammatory activity of the plant leaves, bark and their fractions has been evaluated via formalin induces paw thickness using Vernier caliper. All the extracts showed dose dependent inhibitory activity in formalin induce inflammation over a period of 2.5 hrs.

The paw volume after administration of formalin was measured in mm. The results show that the crude methanolic extract of *M. buxifolia* leaves significantly reduced the paw edema at a dose of 500 m/kg. The paw thickness in *M. buxifolia* leaves treated animals was 2.4 mm in first thirty minutes, whereas after 2.5 h the paw thickness was 3.1 mm ($p<0.001$) as compare with negative control 3.5±0.05 mm (Table **1**). The chloroform fraction also significantly prevented the paw thickness at a dose of 500 mg/kg ($p<0.01$). On the other hand, the *n*-hexane and ethyl acetate fraction less significantly prevent what thickness at 500 m/kg (Table **1**).

The anti-inflammatory potential of M. *buxifolia* bark extract was also observed for 2.5 hrs. All the results showed that the crude extracts and their fractions rivers the inflammatory process. The 300 and 500 mg/kg dose significantly reduced paw thickness 2.7±0.05 and 2.6±0.06 mm ($p<0.01$ and $p<0.001$) respectively, while in negative control the paw thickness was 3.5±0.05 mm. Equal paw thickness with *n*-hexane and ethyl acetate fraction was observed (Table **2**). The chloroform fraction also significantly ($p<0.01$) reverse the inflammatory process.

Table 1. Anti-inflammatory effect of *M. buxifolia* crude leaves extract and its fraction.

Sample	Dose (mg/kg)	Paw thickness measurement (mm) at different time interval in min					
		0	30	60	90	120	150
Control (Distilled water)	—	2.4 ± 0.1	2.9±0.1	3.2±0.04	3.4±0.05	3.1±0.03	3.5±0.05
Diclofenac sodium	10	2.4±0.04	2.5±0.07	2.7±0.2	3.0±0.02	2.9.0±0.1	2.6±0.06**
***M. buxifolia* crude extract**	100	2.1±0.1	2.3±0.2	2.7±0.1	3.0±0.03	3.1±0.07	2.8±0.04
	300	2.4±0.01	2.5±0.1	2.8±0.02	3.0±0.04	3.1±0.06	2.8±0.04**
	500	2.3±0.05	2.4±0.07	2.7±0.2	2.9±0.02	2.9.0±0.1	2.5±0.06**
***M. buxifolia n*-hexane fraction**	100	2.2±0.2	2.4±0.1	2.8±0.05	3.2±0.05	3.4±0.02	3.1±0.06
	300	2.1±0.1	2.3±0.06	2.6±0.08	2.8±0.06	3.0±0.07	2.9±0.05*
	500	2.3±0.06	2.4±0.1	2.7±0.07	2.9±0.07	3.0±0.06	2.8±0.04**
***M. buxifolia* ethyl acetate fraction**	100	2.0±0.1	2.3±0	2.5±0.03	2.9±0.07	3.2±0.04	3.0±0.08
	300	2.2±0.04	2.4±0.07	2.6±0.03	2.9±0.08	3.1±0.03	2.8±0.1*
	500	2.2±0.01	2.4±0.03	2.7±0.02	2.8±0.02	3.0±0.04	2.7±0.05**
***M. buxifolia* chloroform fraction**	100	2.3±0.08	2.6±0.07	2.8±0.01	3.0±0.04	3.2±0.05	3.1±0.08
	300	2.3±0.2	2.5±0.04	2.8±0.08	3.0±0.04	3.1±0.06	3.1±0.03
	500	2.1±0.04	2.3±0.06	2.6±0.05	2.9±0.05	3.0±0.08	2.8±0.02**

Values are expressed as mean ± SEM for *n*=6 animals in each group. Statistical significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Table 2. Anti-inflammatory effect of *M. buxifolia* crude bark extract and its fraction.

Sample	Dose (mg/kg)	Paw thickness measurement (mm) at different time interval in min					
		0	30	60	90	120	150
Control (Distilled water)	—	2.4±0.06	2.9±0.05	3.2±0.1	3.4±0.2	3.1±0.1	3.5±0.2
Diclofenac sodium	10	2.5±0.1	2.5±0.07	2.7±0.09	2.9±0.07	2.9±0.05	2.6±0.06‡
***M. buxifolia* crude extract**	100	2.1±0.07	2.3±0.05	2.6±0.04	2.8±0.05	3.0±0.07	2.8±0.06*
	300	2.4±0.03	2.5±0.04	2.7±0.05	2.9±0.06	3.0±0.08	2.7±0.05‡
	500	2.3±0.1	2.4±0.07	2.6±0.09	2.7±0.07	2.9±0.05	2.6±0.06‡‡
***M. buxifolia n*-hexane fraction**	100	2.2±0.03	2.5±0.06	2.8±0.1	3.1±0.07	3.2±0.1	3.0±0.07*
	300	2.1±0.06	2.3±0.07	2.6±0.03	2.8±0.05	3.1±0.05	2.8±0.05*
	500	2.3±0.2	2.5±0.06	2.7±0.03	2.9±0.08	3.0±0.8	2.7±0.05‡
***M. buxifolia* ethyl acetate fraction**	100	2.0±0.02	2.3±0.05	2.5±0.04	2.8±0.08	3.1±0.7	3.0±0.02
	300	2.2±0.03	2.3±0.08	2.5±0.08	2.8±0.03	3.0±0.03	2.8±0.08*
	500	2.2±0.05	2.3±0.02	2.5±0.2	2.7±0.04	2.9±0.1	2.7±0.08‡
***M. buxifolia* chloroform fraction**	100	2.3±0.06	2.6±0.02	2.9±0.08	3.0±0.04	3.1±0.02	3.1±0.04
	300	2.3±0.1	2.6±0.09	2.8±0.1	3.0±0.04	3.1±0.06	2.9±0.06
	500	2.1±0.05	2.3±0.1	2.5±0.08	2.9±0.05	3.0±0.05	2.8±0.07‡

Values are expressed as mean ±SEM for *n*=6 animals in each group. Statistical significance: *p<0.05; ‡ p<0.01; ‡‡p<0.001.

The COX-2 inhibitory potential of various samples of *M. buxifolia* leaves and bark extract showed dose dependent anti-nociceptive activity Figure **4** a and b. In this assay leaves extract of *M. buxifolia* exhibited 9.8±0.8, 20.50±1.1, 31.85±0.4, 44.80±0.9 and 60.0±0.5% inhibition (p<0.01 and p<0.001) at 62.5, 125, 250, 500 and 1000 µg/ml. The chloroform, *n*-hexane and ethyl acetate fraction of *M. buxifolia* leaves at 1000 µg/ml showed 42.80 ±0.3, 30.97±1 and 29.90±0.28 % inhibition Figure **4a**. The bark crude extract of *M. buxifolia* inhibited 11.77±0.81, 23.43±1.5, 34.8±1.5, 48.5±0.59 and 63.83±0.73 % (p<0.01 and p<0.001) COX-2 at 62.5, 125, 250, 500 and 1000 µg/ml. The chloroform, *n*-hexane and ethyl acetate fraction of *M. buxifolia* bark at 1000 µg/ml showed 43.83 ±0.33, 32.97±1.3 and 31.9±0.78 % inhibition Figure **4b**.

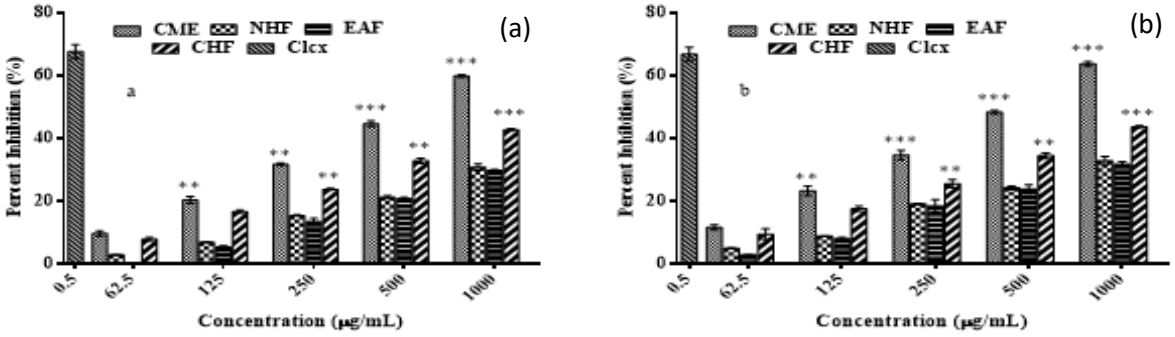

Figure 4. COX-2 inhibitory potential of various samples of *M. buxifolia* (a) leaves (b) bark. Values are expressed as mean ±SEM, n=3, Celcx= celecoxib, *p<0.05; ** p<0.01; ***p<0.001.

DISCUSSION

The leaves and bark crude methanolic extract of *M. buxifolia* and their fractions were evaluated for analgesic effect against acetic acid induced visceral pain. The use of acetic acid for provoking writhing is the simplest and common method for screening of analgesic drugs [18]. The characteristics of pain activity generated by intra-peritoneal injection of acetic acid is presented by abdominal muscles contraction, hind limbs extension and body elongation due to local peritoneal receptors mediation [19]. Acetic acid peripherally liberates endogenous substances and pain mediators like prostaglandins a metabolites of arachidonic acid by cyclooxygenase pathway [20], that cause pain. Broadly it was observed that the total time duration of abdominal stretching taken by individual animal and total no of frequency of stretching by individual animal was reduced in both leaves and bark extracts and their fractions of *M. buxifolia*. However, *M. buxifolia* bark extract more significantly ($p<0.001$) reduced the writhing frequency as compare to leaves extract of *M. buxifolia*. Among fractions the chloroform fraction of *M. buxifolia* bark extract comparatively more significantly ($p<0.001$) reduces the frequency and duration of pain at 500 mg/kg dose, while moderately ($p<0.01$) reduced the writhing frequency or pain at 300 mg/kg dose. The ethyl acetate fraction of *M. buxifolia* bark and leaves moderately reduced pain at 500 mg/kg ($p<0.01$). The results were compared with the negative control i.e. treated with normal saline and positive control diclofenac sodium. The crude methanolic extract of *M. buxifolia* leaves and bark at higher dose significantly inhibited the frequency of writhing and were comparable with standard drug diclofenac. These results are in accordance with the analgesic effect of the fruit extract of *M. buxifolia* which was previously reported by [9].

The anti-nociceptive and anti-inflammatory effect of crude extract of leaves and bark of *M. buxifolia* were also evaluated by formalin induced paw method. Formalin induces inflammation and pain by stimulating inflammatory and pain mediators. This pain model has been considered as a simple unique technique as it consists of 2 phases, i.e. phase-1/or early phase neurogenic phase while phase-2/or late phase is inflammatory phase. In both phases the mice experienced to have pain [16]. The licking and biting characteristic of inflamed paw explain the level of pain. The biphasic biting response was initiated by 2 % formalin administration. The animals administered with crude extract of *M. buxifolia* bark spent significantly ($p<0.001$) less time on licking and biting as compared to *M. buxifolia* leaves extract in the early phase. In the late phase the animals spent more time on licking and biting at ($p<0.01$) with leaves and bark extract. Among fraction of leaves and bark the chloroform fraction showed promising results ($p<0.001$) in early phase, while in the late phase the biting and licking was moderately ($p<0.01$) reduced. The *n*-hexane and ethyl acetate fraction of *M. buxifolia* bark and leaves also diminished significantly ($p<0.01$) the licking and biting response at 500 mg/kg in the early phase. The bark extract *n*-hexane and ethyl acetate fraction results were comparable to positive control diclofenac.

Inflammation is an acute response exhibited by living tissue as a result of any kind of injury. It is a part of body's natural defense mechanism and immunity [21]. The integral task of inflammatory response includes the localization and eventually elimination of harmful substances, secondly the eradication of damaged tissue components resulting in the culmination of healing process of affected tissues, organ and/or organ systems [22, 23, 24]. The anti-inflammatory effect of crude extracts of leaves and bark and their fractions were observed by measuring paw thickness for 2.5 hrs. In the initial 90 minutes the thickness of the mice paw was continuously increase in bark extract. After 90 minutes the gradual increasing in paw thickness was controlled by oral administration of *M. buxifolia* bark extract in a dose dependent manner. At 300 and 500 mg/kg dose of *M. buxifolia* crude methanolic bark extract reduced the paw thickness at level of significance of ($p<0.01$) and ($p<0.001$) respectively. Among fractions the chloroform fraction have significant anti-inflammatory response, and revert the inflammatory process after 150 minutes at ($p<0.01$). The leaves crude extract of *M. buxifolia* also reduced the inflammatory process significantly ($p<0.001$) at 500 mg/kg. The chloroform, *n*-hexane and ethyl acetate fraction also reduced inflammatory process at ($p<0.01$). In the inflammatory process the initial inflammation within an hour of formalin injection may be due to the trauma at the site of injection followed by histamine and serotonin release at the site of injection [25]. In the second phase the inflammation is mediated by cyclooxygenase pathway. So according to this phenomenon in the first phase (1st hour) there was no inhibition of

inflammation. In the second phase the attenuation in inflammation may be due to presence of compounds like botulin in the extract that possibly inhibit inflammatory mediators and reduced inflammation. The anti-inflammatory compounds decreased inflammation by lipopolysaccharide (LPS)-triggered inflammatory system and COX-2 downregulation [26]. Vitamin E identified in the leaves and bark extracts have anti-inflammatory effect by inhibiting prostaglandins and leukotrienes but not inhibiting COX-2 [27]. So according these results the inflammation attenuate in the second phase, probably either by inhibiting COX pathway or inhibiting COX pathway metabolites. Moreover, the anti-inflammatory and anti-nociceptive response of *M. buxifolia* fruit was also previously reported by [9], which support the anti-inflammatory and analgesic effect produced by leaves and bark extract of *M. buxifolia*. However, the anti-inflammatory and analgesic effect of *M. buxifolia* bark extract and its chloroform fraction was more than the methanolic extract of *M. buxifolia* leaves and its fractions. The *M. buxifolia* leaves and bark extract and their fractions were further evaluated via *in vitro* COX-2 enzymatic pathway. The results showed that *M. buxifolia* bark and its chloroform fraction more significantly inhibited COX-2 enzyme as compare to *M. buxifolia* leaves extract.

CONCLUSION

In conclusion, this study demonstrated the significant dose-dependent anti-inflammatory activity of *M. buxifolia* leaves and bark extracts which could be exploited in the search for plant-based anti-inflammatory agents. Based on our current results it is concluded that *M. buxifolia* leaves and bark are rich source of biologically active molecules. The significant results of *M. buxifolia* bark extract and its chloroform fraction demonstrated that it should be the part of complementary and alternative medicine. Present study opens a new window for future investigation on *M. buxifolia* leading to the development of an herbal therapeutic agent which may be of value in inflammatory diseases. Further, it is recommended to isolate and mechanistically investigate the essential oils, secondary metabolites and other bioactive compounds from this plant having potential to combat nociception and inflammation.

REFERENCES

1. Gilroy DW, Lawrence T, Perretti M, Rossi AG. Inflammatory resolution: new opportunities for drug discovery. Nat Rev Drug Discov. 2004; 3: 401.
2. Serhan CN, Chian GN, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008; 8: 349.
3. LouatI K, Berenbaum F. Fatigue in chronic inflammation-a link to pain pathways. Arthritis Res Ther. 2015; 17: 254.
4. Hosseini M, Taiarani Z, Hadjzadeh MAR, Salehabadi S, Tehranipour M, Alaei HA. Different responses of nitric oxide synthase inhibition on morphine-induced antinociception in male and female rats. Pathophysiology. 2011a; 18: 143-149.
5. HosseinI M, Taiarani Z, Karami R, Abad AA NK. The effect of chronic administration of L-arginine and L-NAME on morphine-induced antinociception in ovariectomized rats. *Indian J Pharmacol.* 2011b; 43:541.
6. Karami R, HosseinI M, KhodabandehloO F, KhatamI L, Taiarani Z. Different effects of L-arginine on morphine tolerance in sham and ovariectomized female mice. J. Zhejiang Univ. Sci. B. 2011; 12: 1016-1023.
7. Ripathi KD. Essentials of medical pharmacology, JP Medical Ltd. 2009; 5th ed: 453–68.
8. Vishal V, Sharm AG, Mukesh G, Ranjan B. A review on some plants having anti-inflammatory activity. J Phytopharmacol. 2014; 2: 214-221.
9. Ullah I, Khan JA, Shahid M, Khan A, Adhikari A, Hannan PA, Javed I, Shakeel F, Farooq U. Pharmacological screening of *Monotheca buxifolia* (Falc.) A. DC. for antinociceptive, anti-inflammatory and antipyretic activities. *BMC Complement* Altern Med. 2016b; 16: 273.
10. Jan S, Khan MR, Rashid U, Bokhari J. 2013. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. *Osong Public Health* Res Perspect. 2013; 4: 246-254.
11. Jan S, Khan M R. Protective effects of *Monotheca buxifolia* fruit on renal toxicity induced by CCl_4 in rats. *BMC Complement* Altern Med. 2016; 16: 289.
12. Ullah I, Khan JA, Iqbal Z, Hannan PA, Nasir , Muhammad S, Jahan S, Rehman M. Chemical composition, anti-bacterial and cytotoxic potential of *n*-Hexane soluble fraction of *Monotheca buxifolia* (Falc) A. DC. Fruit. Natl Acad Sci Lett. 2017; 40: 405-408.

13. Burki S, Mehjabeen, Burki ZG, Shah, Z A, Imran M, Khan M. Phytochemical screening, antioxidant and in vivo neuropharmacological effect of *Monotheca buxifolia* (Falc.) barks extract. Pak J Pharm Sci. 2018; 31: 1519-1528.
14. Burki S, Burki ZG, Jahan N, Muhammad S, Mohani N, Siddiqui FA, Owais F. GC-MS profiling, FTIR, metal analysis, antibacterial and anticancer potential of Monotheca buxifolia (Falc.) leaves. Pak J Pharm Sci. 2019; 32(5)(Special): 2405-2413
15. Franzotti E, Santos C, Rodrigues H, Mourao R, Andrade M, Antoniolli A. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L.(Malva-branca). J Ethnopharmacol. 2000; 72: 273-277.
16. Sulaiman M, Hussain M, Zakaria Z, Somchit M, Moin S, Mohamad A, Israf D. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. Fitoterapia. 2008; 79: 557-561.
17. BurnetT, B Jia, Q ZhaO, Y & Levy R. A medicinal extract of Scutellaria baicalensis and Acacia catechu acts as a dual inhibitor of cyclooxygenase and 5-lipoxygenase to reduce inflammation. J Med Food. 2007; 10 : 442-451.
18. Collier H, Dinneen L, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol. 1968; 32: 295-310.
19. Bentley G, Newton S, Starr J. Studies on the antinociceptive action of α-agonist drugs and their interactions with opioid mechanisms. Br J Pharmacol. 1983; 79: 125-134.
20. Lu TC, Ko YZ, Huang HW, Hung YC, Lin YC, Peng WH. Analgesic and anti-inflammatory activities of aqueous extract from *Glycine tomentella* root in mice. J Ethnopharmacol. 2007; 113: 142-148.
21. Verma S. Medicinal plants with anti-inflammatory activity. J Phytopharmacol. 2016 ;5: 157-159.
22. Barnes PJ. Targeting the epigenome in the treatment of asthma and chronic obstructive pulmonary disease. Proceedings of the American Thoracic Society. 2009; 6: 693-696.
23. Garrett W S, Gordon JI, Glimcher LH. Homeostasis and inflammation in the intestine. Cell. 2010; 140: 859-870.
24. Ahmed AU. An overview of inflammation: mechanism and consequences. Front Biol. 2011; 6: 274.
25. Crunkhorn P, Meacock S. Mediators of the inflammation induced in the rat paw by carrageeninBr J Pharmacol. 1971; 42: 392-402.
26. Ci X, Zhou J, Lv H, Yu Q, Peng L, Hua S. Betulin exhibits anti-inflammatory activity in LPS-stimulated macrophages and endotoxin-shocked mice through an AMPK/AKT/Nrf2-dependent mechanism. Cell Death Dis. 2017; 8: e2798.
27. Reiter E, Jiang Q, Christen S. Anti-inflammatory properties of α-and γ-tocopherol. Mol. Aspects Med. 2007; 28: 668-691.