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Original article

Noscapine hydrochloride (benzyl-isoquinoline alkaloid) effectively prevents protein denaturation through reduction of IL-6, NF-kB, COX-2, Prostaglandin-E2 in rheumatic rats

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ABSTRACT

Noscapine hydrochloride (benzyl-isoquinoline antitussive alkaloid) is an opium derivative and generally used as a cough suppressant. Numerous studies on noscapine hydrochloride have reported that it has potent anti-inflammatory activity. However, the mechanisms by which it exerts an anti-inflammatory function is not well understood. Protein denaturation is the primary step that leads to the organ destruction and permanent arthritic disability. The above-mentioned facts provided the ground to plan this study using different *in-vitro* and *in-vivo* approaches. RT-qPCR and ELISA assays were used to assess the inflammatory markers related to protein denaturation in complete adjuvant persuaded rheumatism in Sprague - Dawley rats. The results were collected as paw volume and body weight changes, arthritic scoring and serum antioxidant enzymes assays. These findings demonstrated that all doses of noscapine hydrochloride (10, 20 and 40 mg/kg) studied in this study, significantly ($p < 0.001$) decreased the protein denaturation by preventing the increase in levels of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), nuclear factor-kB (NF-kB), cyclooxygenase-2 (COX-2) and prostaglandin E2. Noscapine hydrochloride significantly reduced the paw volume ($p < 0.001$), arthritic scoring and reversed the body mass as compared to arthritic control diseased rats.

1. Introduction

Noscapine is a benzyl-isoquinoline alkaloid isolated from *P. somniferum*. From the ancient times, this alkaloid has been used as a cough suppressant. In 1998, potent antitumor effect of noscapine was discovered against lymphoid tumors through binding to the active sites of tubulins and inhibiting the microtubule assembly (Sung et al., 2010). Alkaloids are the secondary metabolites of plant, and possess a variety of pharmacological activities like antidepressant, antitumor, anticholinergic, antiviral, diuretics, analgesics, anti-hypertensive and anti-inflammatory (Souto et al., 2011). Many alkaloids containing compounds possess anti-rheumatic activity like berberine, another bis-benzylisoquinoline alkaloid. It has been widely used as an anti-bacterial, anti-inflammatory and antiarthritic medication for several years. Berberine has a substantial immunomodulatory effect against investigational autoimmune myocarditis (Wang et al., 2017). Other isoquinoline alkaloids like columbamine, palmatine and berbamine showed inhibitory effect in hind paw edema induced by

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serotonin. Quinolizidine alkaloids like oxymatrine and matrine exhibited antioxidant effect, and also inhibit the cyclooxygenase pathway (Souto et al., 2011).

Rheumatoid arthritis (RA) is a multifactorial chronic infectious disorder that affects 0.5–1.0 % of peoples in the world. It starts with inflammation of synovial hyperplasia, synovial proliferation and development of cartilage, ultimately causes pain, swelling and functional disability of joints (Scott, 2010). If RA is left untreated, 20–30 % of patients can become permanently disabled (Rindfleisch and Muller, 2005). Pro-inflammatory cytokines such as TNF-α, IL-1β induce the release of other cytokines, whereas, IL-6 activates the production of acute phase protein reaction (Bellingan, 1999). These cells are involved in the expression of COX-2 and production of PGE2. Certain endogenous substances downregulate the inflammatory reactions by inducing protective mechanisms against inflammation. Specific cytokines such as IL-4 down-regulates the LPS induced expression of COX-2 and inhibits the macrophage response. Similarly IL-10 inhibits the adverse outcomes of stimulated inflammatory cells by preventing the release of IL-6, TNF-α, IL-8 and IL-1β (de Cássia et al., 2015).

Furthermore, lymphocytes and macrophages in the synovial fluid of arthritic joint induces the production of reactive oxygen and nitrogen species (ROS and NS) by reperfusion of swollen joints (Cedergren et al., 2007). Oxidative proliferation of proteins and phospholipids increases their permeability in the synovial membrane which initiates the discharge of pro-oxidant and pro-inflammatory mediators into the blood resulting in the interruption of redox signaling pathways. These outcomes induces the oxidative stress, and induces tissue damage (Sarsour et al., 2009).

Currently synthetic drugs are the first line drugs used to manage the RA. Whereas the conventional management of RA includes the use of DMARDs, NSAIDs, analgesics, corticosteroids and biological modifiers like IL-1 receptor blockers. Due to high cost of currently available therapies and their untoward effects, there is a great need to discover new effective medications against RA which cost less and have minimum side effects. Ayurveda provides the detail for anti-inflammatory natural compounds against arthritic disorder. Therefore it is of great importance to find out the anti-arthritic agents from natural sources with immense antioxidant potential (Bag et al., 2013). The present study is the first study of its kind that studies the antiarthritic effect of noscapine hydrochloride (NH) by using in-vitro and in-vivo methods.

2. Materials and methods

2.1. Experimental animals

Male Sprague Dawley healthy rats weighing between 150 and 300 g were used in this study. All animals were kept under controlled photoperiod (12:12 h) dark: light cycles, relative humidity (50-60°) and environmental temperatures (24–26 °C). They received standard pellet diet and water. All experimental procedures related to handling of rats were approved by the Animal Ethical and Review Committee at college of Pharmacy, University of Sargodha (Approval NO. UOS/Pharm/Animal Ethics −2015/2019).

2.2. Drug

Noscapine hydrochloride (Rhawn, China), Catalog number (912–60--7 ≥ 98 %), molecular weight (449.9 g/mole) and molecular formula ($C_{22}H_{24}ClNO_7$) was used for this study.

2.3. In-vitro anti-rheumatic activity

2.3.1. Reduction in protein denaturation by egg albumin

Initially, 5 mL of total assay mixture contained 0.20 mL of egg albumin, 2.80 mL of phosphate buffer and 2.0 mL of different concentrations of noscapine hydrochloride (6400-50ug/mL) was used in assay. The resulting mixtures with different NH concentrations were incubated for 15 min at 35–39 °C. Then mixture was heated for 5 min at 70 °C. Cooled the mixtures at room temperature and absorbance was observed at 660 nm by using UV Spectrophotometer (Hasan et al., 2015). The percentage reduction in protein denaturation was calculated by using the formula.

$$\text{Percent reduction} = (\text{Abs. of Tc - Abs of Ts}/\text{Abs of Tc}) \times 100$$

Abs. absorbance, Tc is test control and Ts is test solution.

2.3.2. Reduction of protein denaturation by BSA

Here, 0.5 mL of test solution contained 0.45 mL of bovine serum albumin (5 %) and 0.05 mL of different NH concentrations (6400–50 μg/mL). 0.5 mL of product control solution contained different concentrations of NH (6400–50 μg/mL) and 0.45 mL of distill water. pH of the aforementioned solutions was calibrated to 6.30 with 1 N HCl. Then treatment solutions were kept at 37 °C for twenty minutes and then heated for thirty minutes at 57 °C. Afterwards, these were cooled and then added with 2.50 mL of phosphate buffer saline (pH 6.30) in each test tube. Then absorbance was measured at 660 nm (Hasan et al., 2015). Percent reduction in protein denaturation was calculated by using the formula given below.

$$\text{Percent reduction} = [100 - (\text{Abs of Ts} - \text{Abs of Pc}/\text{Abs of Tc}) \times 100]$$

Abs = absorbance, Ts = test solution, Pc = product control and Tc = test control.

2.3.3. HRBC membrane stabilization method

Blood samples were taken from healthy human volunteers and assorted with Alsever's solution. Centrifugation was done for 15 min at 3000 rpm in a centrifuged machine. Pack cells were isolated and washed with 10 % v/v isosaline solution. 10 % suspension of red blood cells was made with isosaline solution (10 % v/v). Test solution contained 1.0 mL of phosphate buffer, 2.0 mL of hypotonic solution, 0.50 mL of RBCs suspension and 0.50 mL of different concentrations of NH (6400, 3200, 1600, 800, 400, 200, 100 and 50 μg/mL), and naproxen used as a standard drug respectively. Then these solutions were incubated for thirty minutes at 37 °C. Centrifugation was done for the solutions for 5 min at 3000 rpm. Poured out the supernatant liquid and absorbance was measured by using UV/visible spectrophotometer at 560 nm. Percent protection was measured by using the following formula (Hasan et al., 2015).

$$\text{Percent protection} = 100 - (\text{Abs of Ts}/\text{Abs of Tc}) \times 100$$

2.4. In-vivo anti-rheumatic activity

2.4.1. Formaldehyde persuaded arthritis in Sprague Dawley rats

The rats were divided into different groups including five rats in each group. Group I was designated as arthritic disease control group and received only purify water through oral gauge (10 mL/kg) per day. Group II, III, IV, V served as treatment groups and received naproxen 20 mg/kg, noscapine hydrochloride 10, 20 and 40 mg/kg. Drug was administered by oral route for the period of 10 days. At day 1 and 3, arthritis was induced by subcutaneous injection of formaldehyde in the left hind foot of each rat. Paw volume was measured by using digital plathysmometer and

compared the treatment group with diseased untreated control group by using the formula given below (Akhter et al., 2021).

$$\text{Percent inhibition} = (Vc - Vt/Vc) \times 100$$

2.4.2. Complete adjuvant persuaded rheumatoid arthritis in Sprague Dawley rats

The animals were randomly separated into six groups (n = 5). Group I served as a normal untreated control group and received only 3 mL/kg of distill water (Complete adjuvant not injected). Group II served as rheumatoid arthritis control group and received only 10 mL/kg distilled water orally. Group III was treated with naproxen sodium 20 mg/kg. Group IV, V and VI served as treatment groups and received noscapine hydrochloride 10, 20 and 40 mg/kg respectively. Drugs were administered by oral route through gastric intubation daily for the period of 28 days respectively. At day 0, immunological rheumatoid arthritis was induced by injecting 0.1 mL of Freund's complete adjuvant into the left footpad of each animal 30 min after administration of treatments except group I. On day 28, the rats were anesthetized with pentobarbitone (6 mg/100 g), and blood and serum samples were collected along with ankle joints of treated and untreated rats. Arthritis was evaluated by assessing various parameters.

2.4.3. Evaluation of arthritis through physical parameters

Body weight of each rat was measured weekly throughout the treatment period. Paw volume was determined on day 0, 7, 14, 21 and 28th of treatment by using digital plathysmometer. The percent suppression of paw volume was calculated on similar day (Akhter et al., 2022).

2.4.4. Evaluation of arthritis from arthritic development (scoring)

The severity and induction of arthritis was evaluated by visual arthritic scoring method at day 0, 7, 14, 21 and 28 days of treatment. Arthritic score for a single paw ranged from 0 to 4, where 0 score designates paw of normal rat, while score 1–4 describes edema and erythema of 1 paw/number to involvement of all digits and whole paw. Score 5 or above presents swelling in complete leg, and inability to twist it correspondingly (Shabbir et al. 2016).

2.4.5. Evaluation of arthritis from hematological & biochemical parameters

Blood parameters including ESR, RBCs, platelets count, WBCs and hemoglobin contents were evaluated by using automatic hemocytometer (sysmex-XT-18000i). Commercially available kits were used for the determination of kidney function test including creatinine and RF at pathological laboratory in Diagnostic Center of University of Sargodha, Sargodha Pakistan (Akhter et al., 2022).

2.4.6. Evaluation of pro-inflammatory expression levels (IL-1β, TNF-α AND IL-6), inflammatory (NF-kB, COX-2 and PGE2) and anti-inflammatory mediators (IL-4 and IL-10) by using RT-qPCR

Total RNA extraction was done from blood samples by following the TRIzol method. Quantification of RNA and its yield was determined by using nanodrop spectrophotometer. From total, RNA 500 ng/reaction of the RNA was used to make cDNA by using commercially available kit and following the manufacturer protocol (Gene Direx). The cDNA template was amplified with 45 cycles of denaturation for 10 *sec* at 95 °C, annealing phase was for 20 s at 60 °C and extension phase for 30 s at 72 °C. The Bio-Red system was used to perform the RT-qPCR. Gene specific primers along with amplified base pair are given in Table 4, where GAPDH was used as reference gene (Akhter et al., 2022).

2.4.7. Evaluation of serum prostaglandin E_2

On day 28 after complete adjuvant immunization, blood samples were collected by cardiac puncture and allowed to clot for 01:00 h. Serum was obtained by centrifugation for 5 min at 3000 rpm and stored at −20 °C. The level of prostaglandin E_2 was determined in serum using ELISA kit for rat prostaglandin E_2 having (Cat No. E0504Ra, Bioassay technology laboratory technology). ELISA plate was read at 450 nm using micro plate spectrophotometer (Akhter et al., 2022).

2.4.8. Evaluation of antioxidant enzymes (CAT, POD, SOD)

The catalase test was used to evaluate its capacity to decrease the H_2O_2 at wavelength 240 nm. Test solution contained 3.0 mL of potassium phosphate buffer (50 mM) at pH 7, 0.1 mL of enzyme extract and 0.1 mL of 30 Mm of hydrogen peroxide. After three minutes, optical density was measured at 240 nm (Akhter et al., 2022) and activity of Catalase was calculated by given formula.

$$\text{Catalase activity} = (A3/0.04 \times 0.01) \times 3$$

Whereas; A3 = Absorbance at 240 nm, 0.04 = Extinction coefficient for H2O2 ($M^{-1}CM^{-1}$).

Peroxidase (POD) activity was evaluated to check its ability to reduce hydrogen peroxide at 470 nm (Akhteret al.2022). Assay mixture was comprised of buffer substrate solution (3 mL) and 0.06 mL of enzyme. The above solution was added into the mixture of 0.32 mL of Hydrogen peroxide, 0.7 mL of guaiacol and 47 mL of 0.2 M phosphate buffer. After 3 min of reaction, absorbance was observed through spectrophotometer against blank at 470 nm.

POD bustle was calculated by using given formula.

$$\text{POD bustle (U/ML)} = \frac{A}{26.6 \times 3.0 \times 0.06}$$

Whereas; A = Absorbance at 470 nm, 26.6 = Guaiacol extinction coefficient ($Mm^{-1}cm^{-1}$ 0.06 = size of enzyme extract (mL), 3.0 = size of phosphate buffer (mL).

The superoxide dismutase (SOD) assay was done by following protocol as established by Worthington 1988 with slight modifications. Assay mixture contained 0.05 mL of enzyme extract, 1 mL of potassium phosphate buffer solution at pH 7.8 and 0.016 mL of riboflavin solution (0.012 mM). The mixture was incubated for 12 min in a light box. After this, we added 0.067 mL of NaCN/EDTA solution and 0.033 mL of nitro-blue tetrazolium solution. After 30 s, absorbance was measured by spectrophotometer against blank at wavelength of 560 nm (Akhter et al., 2022). The SOD activity was measured by using the formula given below.

$$\text{Percentage inhibition} = A\ (\text{Blank})A(\text{Sample})/A\ (\text{Blank}) \times 100$$

2.5. Statistical analysis

Values were displayed as a mean ± SEM. One-way and two-way analysis of variance (ANOVA) was used for the statistical analysis, and GraphPad Prism was used for the Bonferroni posttest. Confidence level was rated as ^{***,c} $p < 0.001$, ^{**,b} $p < 0.01$, ^{*,a} $p < 0.05$ and ^{ns} non-significant.

3. Results

3.1. Effect of noscapine hydrochloride on reduction of protein denaturation (egg albumin, BSA in-vitro assay)

Table 1 shows that noscapine hydrochloride exhibited strong reduction of protein denaturation induced by egg albumin in a concentration dependent manner and ranged from 29.3 to 83.18 %. The effect of noscapine hydrochloride against protein denaturation was not increased when concentration was doubled from 800 µg/mL to

Table 1
In-vitro analysis of noscapine hydrochloride effects on percentage reduction of protein denaturation by egg albumin, BSA, and by HRBC membrane stabilization method.

Treated groups	50 μg/mL	100 μg/mL	200 μg/mL	400 μg/mL	800 μg/mL	1600 μg/mL	3200 μg/mL	6400 μg/mL
Percentage reduction of protein denaturation by egg albumin								
Naproxen	26.63 ± 0.60	35.71 ± 0.1	42.40 ± 0.9	49.61 ± 0.37	63.12 ± 0.63	68.83 ± 0.62	73.59 ± 0.15	80.29 ± 0.29
Noscapine HCl	29.3 ± 0.35^{b}	39.54 ± 0.36^{c}	50.98 ± 0.51^{c}	57.49 ± 0.89^{c}	73.01 ± 0.15^{c}	76.01 ± 0.90^{c}	80.37 ± 0.33^{c}	83.18 ± 0.15^{b}
Percentage reduction of protein denaturation by BSA								
Naproxen	20.88 ± 0.05	24.51 ± 0.13	32.68 ± 0.23	39.92 ± 0	45.78 ± 1.58	55.54 ± 0.63	68.23 ± 0.29	83.49 ± 0.22
Noscapine HCl	20.31 ± 0.90 ns	28.89 ± 0.05^{c}	36.13 ± 0.39^{b}	42.80 ± 0.32^{a}	49.15 ± 0.39^{b}	64.21 ± 1.30^{c}	73.87 ± 0.28^{c}	89.57 ± 0.38^{c}
Percentage reduction of protein denaturation by HRBC membrane stabilization method								
Naproxen	13.57 ± 0.16	18.11 ± 0.33	23.87 ± 0.05	32.31 ± 0.49	45.47 ± 0.24	49.67 ± 0.28	53.30 ± 0.05	69.30 ± 0.31
Noscapine HCl	14.40 ± 0.28 ns	20.05 ± 0.03^{b}	27.94 ± 0.03^{c}	37.19 ± 0.28^{c}	48.15 ± 0.34^{c}	54.25 ± 0.30^{c}	63.64 ± 0.21^{c}	75.29 ± 0.97^{c}

Values are summarized as (mean ± S.E.M, n = 3) using the Dunnet postt-test followed by one-way ANOVA. ^{ns} presents non-significant, while ^{c} $p < 0.001$, $^{b}p < 0.01$, and $^{a}p < 0.05$ against the arthritis control group.

1600 μg/mL, and maximum effect was achieved against thermal induced denaturation at 6400 μg/mL as compared to naproxen (80.29 %) at same concentrations.

Similarly effect of noscapine against heat induced denaturation of protein by BSA was almost same as discussed in egg albumin protein denaturation. But they showed continuous increased in inhibition in the concentration dependent manner. The effect against thermally induced denaturation of protein was ranged from 20.31 to 89.57 %. At initial concentrations, effect of noscapine hydrochloride was similar with respect to naproxen, but as the concentration was increased the effect of noscapine hydrochloride (4–5 % almost) was increased as compared to naproxen at same concentrations.

3.1.1. Effect of noscapine hydrochloride on HRBC membrane stabilization method

Noscapine hydrochloride significantly protected the membrane from hemolysis in HRBC stabilization method in a dose-dependent manner. The percent protection against lysis was ranged from 14.40 to 75.29 %. The stabilization of membrane by noscapine hydrochloride was 6 times greater than the protection provided by naproxen, as illustrated in Table 1.

3.2. In-vivo anti-rheumatic activity

3.2.1. Effect of noscapine hydrochloride on formaldehyde induced arthritis

After induction of arthritis by formaldehyde on day 1 and 3, it expressed paw swelling of each rat. Paw edema was increased on day 6 (2.00 ± 0.07) in arthritic untreated rats in contrast to rats treated with noscapine hydrochloride 0.73 ± 0.01, 0.67 ± 0.01 and 0.69 ± 0.00 at doses of 10, 20, and 40 mg/kg respectively. Maximum inhibitory effect (71.81 %) was achieved on day 10 against paw volume by noscapine hydrochloride at 20 mg/kg dose. Initially, lowest and highest doses of noscapine hydrochloride showed dose dependent inhibitory effect against paw volume from day 2 to day 6 but effect did not increase prominently from day-8 to day-10. On day-10, NH was six times more effective than naproxen sodium against rheumatism as shown in Fig. 1.

3.2.2. Effect of noscapine hydrochloride on parameters of arthritis (paw volume, body weight)

Paw edema was observed after induction of arthritis with CAI. Oral administration of noscapine hydrochloride showed significant dose dependent reduction in paw volume throughout the treatment. A slight difference was observed on day 2nd that noscapine hydrochloride (20 mg/kg) was 2 times more effective than 40 mg/kg for the same day of treatment. Maximum reduction in paw volume (68.42 %) was observed with NH at 80 mg/kg on day 28. As treatment continued, the drug showed similar results at 40 mg/kg dose on day 21st and 28th with slight changes, proved to be more effective at dose 20 mg/kg than higher doses. At last day of treatment on similar doses, noscapine hydrochloride (63.15 %) was 12.44 % stronger than naproxen sodium against inhibition of paw volume (50.71 %) as in Table 2.

Body mass of each rat was recorded on day 0, 7th, 14th, 21st and 28th of treatment. The rise in body mass was perceived in groups treated with NH at 10, 20 and 40 mg/kg as compared to arthritic rheumatoid controlled rats, as shown in Fig. 2.

3.2.3. Effect of noscapine hydrochloride on arthritic-development (scoring)

Primary and secondary lesions were observed in treated and non-treated rats after induction with CAI, which were more prominent in arthritic controlled rats (Fig. 3). Inflammatory edema was observed in rats from 4 to 7 days, whereas arthritic lesions in non-treated rats (arthritic control) were seen from 10 to 28 days. Treated rats showed significant ($p < 0.001$) reduction in arthritic scoring either by decreasing the paw swelling or inflammatory signs (3.8 ± 0.37, 3.4 ± 0.51 and 3.8 ± 0.58) at three different doses respectively on last day of treatment as compared to arthritic control group (11.8 ± 1.3).

3.2.4. Noscapine hydrochloride normalized the biochemical and hematological parameters

Hematological and biochemical profiles of Sprague Dawley rats treated with noscapine hydrochloride and naproxen sodium are given in Table 3. A decrease in the level of hemoglobin and red blood cells, whereas rise in the levels of platelets, ESR and WBCs were observed in diseased control groups. No such fluctuations were seen in normal groups of rats. Treatment with NH significantly reversed the hematological variations closed to normal control rats induced by inoculation of complete adjuvants injection into the left footpad. Moreover, the tested drug significantly ($p < 0.001$) reduced the level of AST and ALP which were significantly raised in arthritic control rats whereas it decreased, though not significant, the level of ALT as compared to arthritic control animals. Furthermore, noscapine hydrochloride normalized the elevated levels of urea and creatinine as compared to arthritic rats. These outcomes suggested that at test doses noscapine hydrochloride did not show nephrotoxicity and hepatotoxicity. In addition, high RF values were detected in diseased control rats as compared to noscapine treatment groups. Oral administration of test drug significantly decreased the serum RF values in rats treated at 10, 20 and 40 mg/kg.

3.2.5. Noscapine hydrochloride down-regulated the effect of IL-1β, TNF-α, IL-6, COX -2 and NF-kB

A significant up-regulation in the level of IL-1β was perceived in arthritic group (3.96 ± 0.05) as compared to normal animals. Treat-

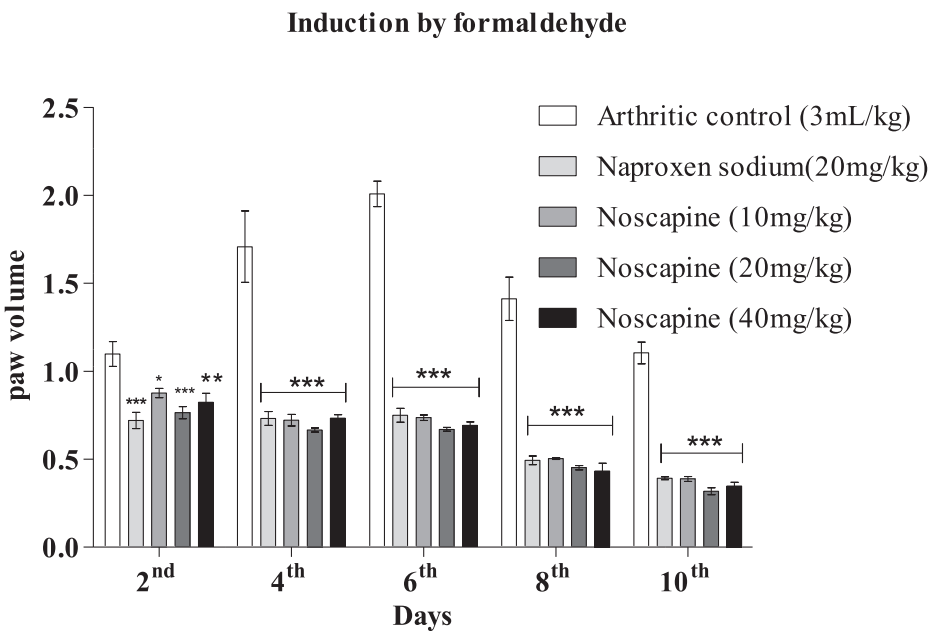

Fig. 1. Effect of noscapine hydrochloride at 10, 20 and 40 mg/kg doses on formaldehyde persuaded arthritis. Results are recorded as (n = 5, mean ± SEM) followed by Two-way ANOVA (*** $p < 0.001$, *$p < 0.05$) statistically significant versus arthritic control.

Table 2
Effect of Noscapine hydrochloride at 10, 20, and 40 mg/kg doses daily for 28 days on paw volume in arthritis persuaded by CFA.

Treatment groups	2nd day	7th day	14th day	21st day	28th day
Upsurge in paw volume					
Arthritic control	1.33 ± 0.24	2.01 ± 0.11	2.5 ± 0.21	2.84 ± 0.21	2.09 ± 0.08
Normal control	0.43 ± 0.04	0.42 ± 0.02	0.37 ± 0.03	0.38 ± 0.03	0.33 ± 0.02
	(67.66 %) [c]	(79.10 %) [c]	(85.20 %) [c]	(86.60 %) [c]	(84.21 %) [c]
Naproxen sodium	1.03 ± 0.05	1.15 ± 0.04	1.11 ± 0.054	1.12 ± 0.08	1.03 ± 0.01
(20 mg/Kg)	(22.55 %)[ns]	(42.78 %) [c]	(55.60 %) [c]	(60.56 %) [c]	(50.71 %) [c]
Noscapine hydrochloride	1.16 ± 0.21	1.44 ± 0.16	1.37 ± 0.06	1.21 ± 0.04	1.02 ± 0.02
(10 mg/kg)	(12.78 %)[ns]	(28.36 %) [c]	(45.20 %) [c]	(57.39 %) [c]	(51.19 %) [c]
Noscapine hydrochloride	1.19 ± 0.16	1.26 ± 0.02	1.31 ± 0.01	1.16 ± 0.01	0.77 ± 0.08
(20 mg/kg)	(10.52 %)[ns]	(37.31 %) [c]	(54.80 %) [c]	(59.15 % [c]	(63.15 %) [c]
Noscapine hydrochloride	0.85 ± 0.13	1.25 ± 0.2	1.13 ± 0.06	1.06 ± 0.04	0.63 ± 0.11
(40 mg/kg)	(36.09 %) [a]	(37.81 %) [c]	(54.88 %) [c]	(62.68 % [c]	(68.42 %) [c]

Values are summarized as (mean ± S.E.M, n = 3) using the Bonferroni post-test followed by two-way ANOVA. [ns] presents non-significant, while [c]$p < 0.001$ and [a]$p < 0.05$ versus the arthritis control group.

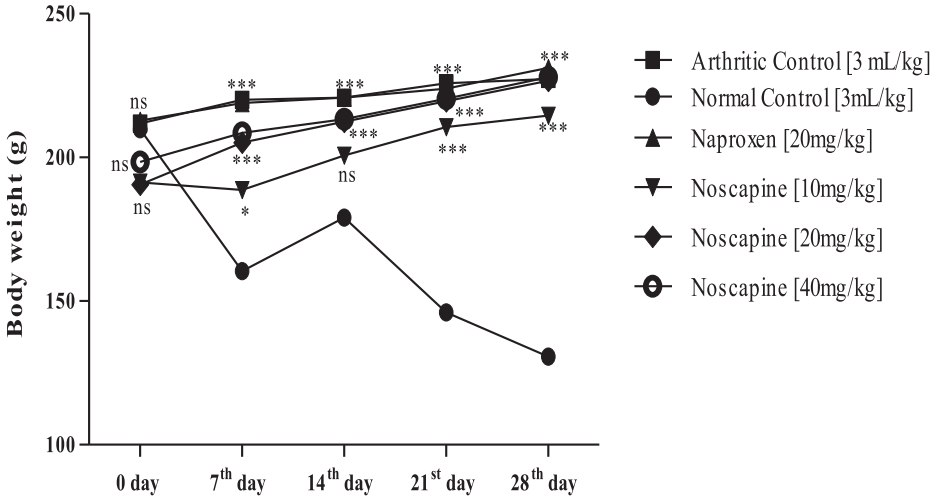

Fig. 2. Effect of noscapine hydrochloride different doses on body mass of rats in CA persuaded arthritis. Two-way ANOVA was done by following Bonferroni post-test while *** $p < 0.001$, *$p < 0.05$ and ns presents non-significant (n = 5), statistically significant versus arthritic control.

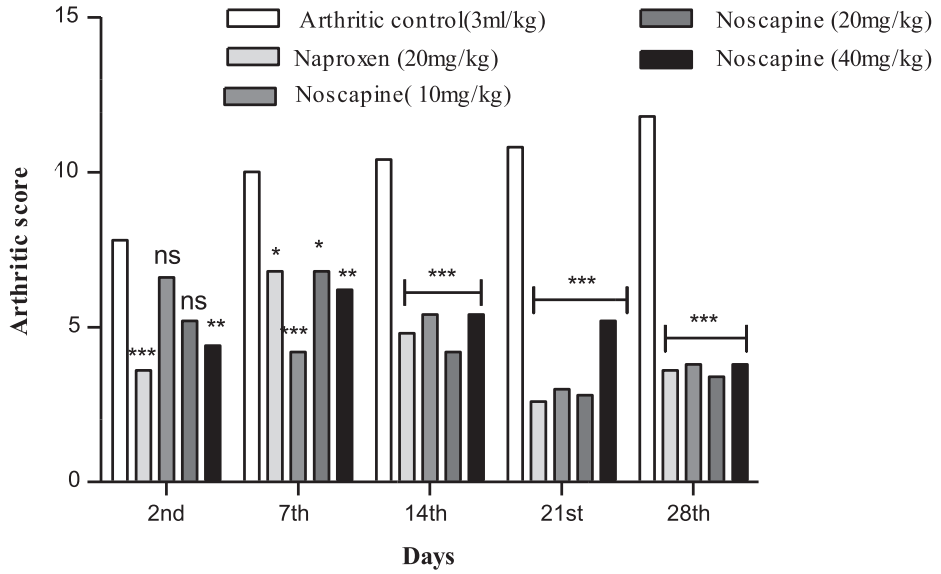

Fig. 3. Effect of noscapine hydrochloride 10, 20 and 40 mg/kg on arthritic scoring of rats in CFA induced arthritis. Two way ANOVA was done by following Bonferroni posttest, whereas *** p < 0.001, ** p < 0.01, * p < 0.05 and ns presents non-significant (n = 5), statistically significant versus arthritic control.

Table 3
Effect of 28-day oral administration of different doses of Noscapine hydrochloride and naproxen on biochemistry parameters versus arthritic control.

Blood Chemistry	Arthritic Control	Normal control	Naproxen sodium (20 mg/kg)	Noscapine HCl 10 mg/Kg	Noscapine HCl 20 mg/Kg	Noscapine HCl 40 mg/Kg
Platelet count	1943.32±	1197.00 ±	1367.81 ±	1438.62 ±	1211.07 ±	1640.67 ±
(10^3/μL)	27.82	40.95^c	72.73^c	25.71^c	19.54^c	14.90^c
WBCs	17.75 ±	9.46 ±	12.48 ±	12.58 ±	11.22 ±	14.10 ±
(10^3/μL)	0.07	0.26^c	0.40^c	0.54^c	0.32^c	0.38^c
ESR	13.88 ±	7.44 ±	10.67 ±	10.65 ±	9.19 ±	12.06 ±
(mm/h)	0.23	0.19^c	0.59^c	0.05^c	0.37^c	0.90 ns
RBCs	3.66 ±	7.07 ±	5.44 ±	6.20 ±	6.98 ±	5.17 ±
((10^6/μL)	0.05	0.22^c	0.07^c	0.04^c	0.11^c	0.38^c
Hb content	10.20 ±	16.74 ±	12.94 ±	11.62 ±	15.97 ±	12.51 ±
(g/dL)	0.15	0.03^c	0.55^c	0.32 ^{a}	0.05^c	0.26^c
ALP	342.28±	212.59 ±	232.12 ±	275.64 ±	224.72 ±	231.30 ±
(U/L)	1.63	1.71^c	1.82^c	0.28^c	0.02^c	2.60^c
AST	79.62 ±	45.88 ±	62.92 ±	64.65 ±	46.03 ±	57.38 ±
(U/L)	0.32	2.01^c	1.55^b	2.85^b	3.78^c	2.58^c
ALT	73.43 ±	40.25 ±	56.98 ±	53.97 ±	41.58 ±	70.34 ±
(U/L)	0.83	0.25^c	5.0^c	1.04^c	0.24^c	0.82 ^{ns}
Urea	51.33 ±	19.44 ±	33.33 ±	47.47 ±	22.31 ±	33.03 ±
(mg/dL)	0.12	0.37^c	0.71^c	1.02 ^{ns}	0.29^c	2.49^c
Creatinine	1.14 ±	0.55 ±	0.82 ±	0.76 ±	0.60 ±	0.85 ±
(mg/dL)	0.09	0.01^c	0.00 ^{a}	0.02^b	0.00^c	0.12 ^{a}
RF	28.21 ±	8.04 ±	13.44 ±	18.61 ±	10.84 ±	15.82 ±
(IU/L)	0.33	0.08^c	1.29^c	0.43^c	0.51^c	0.49^c
CRP	23.44 ±	4.06 ±	12.28 ±	10.32 ±	6.11 ±	9.77 ±
(mg/dL)	0.17	0.06^c	0.36^c	0.27^c	0.01^c	0.17^c

Values are summarized as (mean ± SEM, n = 5) by using ONE- Way ANOVA followed by Dunnet test. ^{ns} presents non- significant, ^{c} p < 0.001, ^{b} p < 0.01 and ^{a}p < 0.05 against the arthritis control group. WBCs = white - blood cells, ESR = erythrocyte - sedimentation rate, RBCs = red - blood cells, Hb = hemoglobin, ALP = alkaline - phosphatase, AST = aspartate - aminotransferase, ALT = alkaline - phosphatase, RF = rheumatoid factor and CRP = C- reactive proteins.

ment with noscapine hydrochloride with 3 different dosses showed a remarkable decrease in the level of IL-1β (2.2 ± 0.05, 1.74 ± 0.04 and 2.62 ± 0.058). The medium dose of noscapine hydrochloride (20 mg/kg) was highly effective against IL-1β (1.74 ± 0.04) as compared to naproxen sodium (2.44 ± 0.121) on same dose level. We also found considerable increase in TNF-α in arthritic control animals (6.12 ± 0.03), while in animal treated with NH exhibited significant reduction in mRNA expression of TNF-α.

Similarly, noscapine hydrochloride and naproxen sodium considerably (p < 0.001) attenuated the level of IL-6 in rats as compared to untreated diseased rats. Similarly, significant increase in level of COX-2 was found in complete adjuvant injected diseased animals (8.4 ± 0.51), that was noticeably reduced by oral administration of noscapine hydrochloride (3.2 ± 0.07, 2.72 ± 0.17 and 3.1 ± 0.03). Likewise, substantial augmentation in the level of NF-kB was detected in arthritic control rats (6.04 ± 0.05). Treatment with noscapine hydrochloride and naproxen sodium significantly reduced the NF-kB expression level (3.06 ± 0.05, 2.74 ± 0.17, 2.56 ± 0.25 and 3.08 ± 0.03) respectively as presented in Fig. 4.

3.2.6. Noscapine hydrochloride up-regulated the expression level of IL-4 and IL-10

A significant increase (p < 0.001) in the level of IL-4 was observed in rats treated with noscapine hydrochloride and

Table 4
Sequences of primers for cytokine's analysis.

IL-4	Forward primers	5-CACCTTGCTGTCACCCTGTT-3	195
	Reverse primers	5-CCTGCAGATGAGCTCGTTCT-3	
IL-10	Forward primers	5-GCCCAGAAATCAAGGAGCAT-3	210
	Reverse primers	5-CGTAGGCTTCTATGCAGTTG-3	
IL-1β	Forward primers	5-GCTGTCCAGATGAGAGCATC-3	293
	Reverse primers	5-GTCAGACAGCACGAGGCATT-3	
IL-6	Forward primers	5-AGACTTCCAGCCAGTTGCCT-3	233
	Reverse primers	5-CTGACAGTGCATCATCGCTG-3	
TNF-α	Forward primers	5-AGGACACCATGAGCACGGAA-3	234
	Reverse primers	5-GGGCCATGGAACTGATGAGA-3	
NF-kβ	Forward primers	5-GCAACTCTGTCCTGCACCTA-3	203
	Reverse primers	5-CTGCTCCTGAGCGTTGACTT-3	
COX-2	Forward primers	5-GCATTCTTTGCCCAGCACTT-3	210
	Reverse primers	5-GTCTTTGACTGTGGGAGGAT-3	
GAPDH	Forward Primers	5-CTTGCCGTGGGTAGAGTCAT-3	229
	Reverse Primers	5-TCTCTGCTCCTCCCTGTTCT-3	

naproxen sodium (61.80 ± 0.86, 76.20 ± 1.53, 68.40 ± 1.24 and 52.20 ± 0.86) as compared to arthritic control (29.53 ± 0.34). Similarly, augmented level of IL-10 was found in rats treated with noscapine hydrochloride (64.00 ± 1.04, 76.00 ± 1.44 and 66.40 ± 1.66), as compared to arthritic control group (41.40 ± 0.74) as shown in Fig. 5.

3.2.7. Effect of noscapine hydrochloride on serum prostaglandin E_2

Noscapine hydrochloride at 10, 20 and 40 mg/kg presented a noteworthy decrease in level of PGE_2 0.71 ± 0.02, 0.62 ± 0.01 and 0.78 ± 0.03 respectively when compared with non-treated adjuvant induced arthritic control groups (1.35 ± 0.019). Noscapine at 20 mg/kg gave (54.07 %) inhibition of PGE_2 as compared to naproxen sodium (39.85 %). So, with same doses, noscapine hydrochloride 26.29 % was more effective than naproxen and endorsed its potent anti-inflammatory effect against rheumatoid arthritis (Fig. 6).

3.2.8. Antioxidant enzyme activity

We further assessed the antioxidant activity level from serum which was collected on 28th day of medication and measured the level of POD, and compared with arthritic control group. Noscapine hydrochloride through oral route increased the antioxidant enzyme activity. Maximum effect was achieved at median dose of NH that was 50 times higher than naproxen sodium dose. The drug increased the defense system of body by increasing the activities of antioxidant enzymes against oxidative stress. These enzymes collectively work and potentiate the effect of each other. Test drug significantly increased the level of SOD, and increased the conversions of superoxide into hydrogen peroxide and oxygen free radicals. Noscapine hydrochloride found to be more effective at 20 mg/kg (26.00 ± 0.37) as compared to other NH doses and naproxen sodium doses. Likewise, the level of CAT was increased in rats treated with noscapine hydrochloride (64.99 ± 0.22, 76.89 ± 0.05 and 20.03 ± 0.91) in comparison to arthritic group (10.45 ± 0.17) presented in Fig. 7.

4. Discussion

Rheumatoid arthritis is a chronic inflammatory disease, and to date, there is no potent treatment for this disease. NSAIDs, corticosteroids, DMARDs and immunosuppressants are used to manage and control the pain and structural abnormalities caused by RA. However these drugs are associated with cost and severe adverse effects (Wang et al., 2017). Noscapine hydrochloride is a natural occurring benzyl-isoquinoline alkaloid phytochemical derived

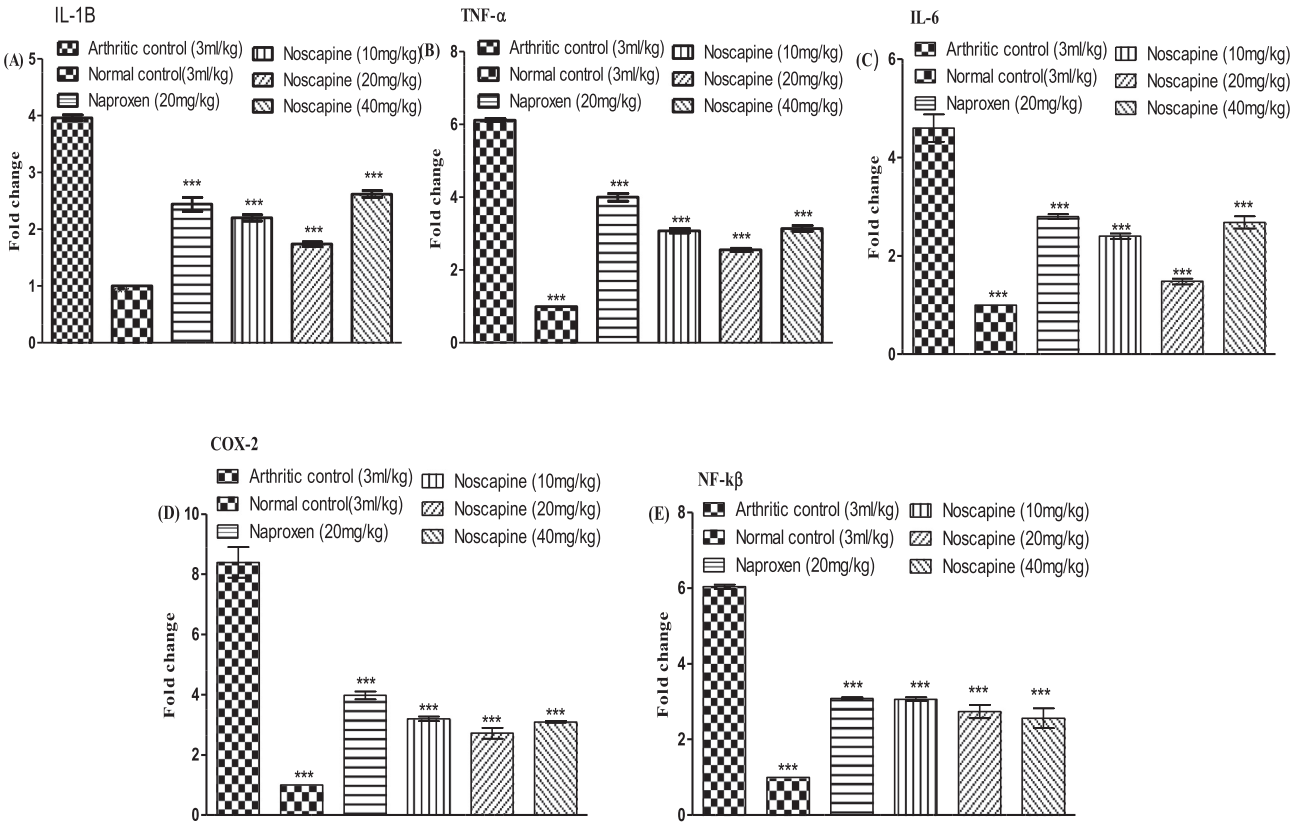

Fig. 4. Effect of Noscapine hydrochloride at increasing doses for 28 days on IL-1β, TNF-α, IL-6, COX-2, NF-kB in CFA-induced arthritic rats. Values are summarized as mean ± S.E.M, using one- way ANOVA followed by Dunnett's post-test (***p < 0.001), statistically significant versus arthritic control.

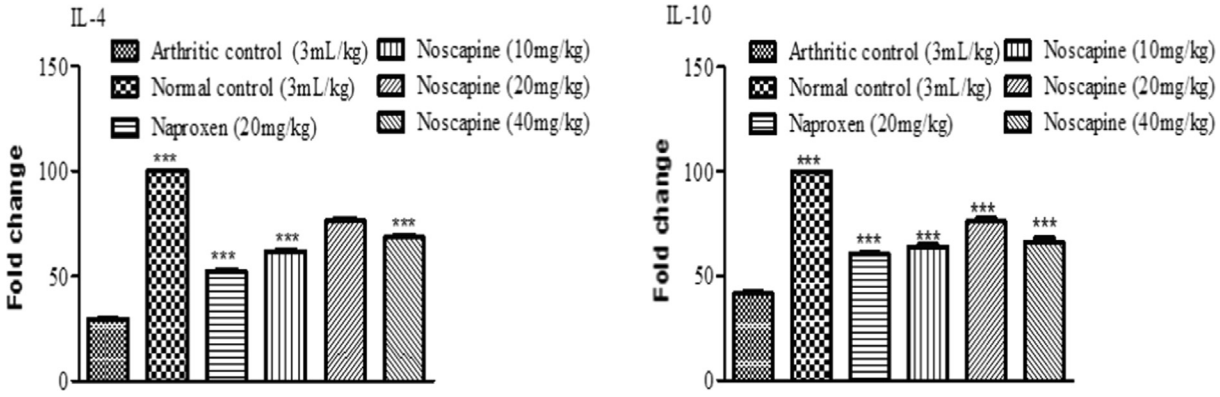

Fig. 5. Augmentation of IL-4 and IL-10 by noscapine hydrochloride (at 10, 20 and 40 mg/kg dose orally for 28 days) in contrast to CFA arthritic diseased control rats. Values are summarized as mean ± S.E.M, using one- way ANOVA followed by Dunnett's post-test (***p < 0.001), statistically significant versus arthritic control.

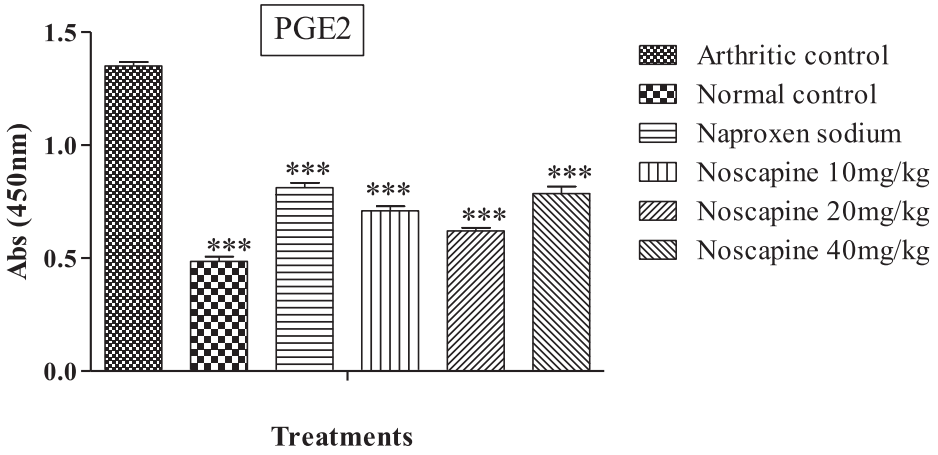

Fig. 6. Effect of noscapine hydrochloride at 10, 20 and 40 mg/kg orally for 28 days on PGE2 in CFA-induced arthritic model versus arthritic control. Results were analyzed by Mean ± SEM followed by one way ANOVA and results were statistically significant where (***p < 0.001).

from *P. somniferum*, has potent anti-inflammatory effect, plenteous in natural herbal sources. So, the current study was designed for first time where noscapine hydrochloride relieved the rheumatism in both *in-vitro* and *in-vivo* studies.

The formation of macroglobulin and protein denaturation can make some proteins auto-antigenic which can initiate the auto-immune reaction that can converge onto the rheumatic arthritis. Auto-antigens formation due to thermally induced protein denaturation (fresh hens egg and BSA) causes the alterations in disulphide, hydrogen and electrostatic hydrophobic bonding (Chandra et al., 2015). In present study noscapine hydrochloride notably reduced the heat induced protein denaturation (fresh hen's egg and BSA). Consequently, ability of noscapine to inhibit the production of auto antigens and protein denaturation may be due to its anti-arthritic effect.

Moreover, exposure of RBCs to detrimental substances such as heat and hypotonic solutions results in membrane lysis due to oxidation of hemoglobin and hemolysis. Since, HRBC membranes resembles closely with lysosomal membranes, so inhibition of thermally induced lysis and hypotonicity was taken as a mechanism of anti-rheumatic drugs. Hypotonic solutions cause the accumulation of fluid and debris within the chamber resulting in membrane rupture. However, lysis of lysosomal membranes cause the tissue damage by lipid peroxidation through influx of free radicals. Stabilization of membranes prevents the leakage of fluid and debris into the tissues during the period of augmented penetrability instigated by inflammatory mediators (Anosike et al., 2012). Noscapine hydrochloride noticeably inhibited the hypotonicity induced lysis, proposes that noscapine hydrochloride inhibits the rupturing of erythrocytic membrane and thus obstructs the tissue impairment instigating by release of enzymes from lysosomes as shown in Table 2. Hence, noscapine hydrochloride stabilizes the RBCs membrane.

Formaldehyde induced arthritis is one of the most customarily used model for evaluating the anti-arthritic activity of certain drugs. After injection of formaldehyde (0.1 mL) in the rat foot induces the paw edema due to the liberation of prostaglandins at injection site. Prostaglandins are the key markers of inflammatory hyperplasia that is arbitrated through activation of nociceptors and hypersensitivity at site of injury (Desai Nilesh et al., 2012). In current investigation, inhibition of paw volume by noscapine hydrochloride in formaldehyde acute model may be due to inhibition of release of prostaglandins at site of inflammation as described in Fig. 2. Moreover, inhibition of prostaglandins also inhibits the cyclooxygenase pathway that supports its antiarthritic activity.

Complete adjuvant is comprised of dried inactivated mycobacterium which is responsible for the activation of cell mediated immunity which eventually augments the generation of certain immuno-globulins. Complete adjuvant induced arthritis is a biphasic chronic arthritis (Walz et al., 1971). Primary phase includes release of prostaglandins while secondary phase includes generation of auto-antibodies. The release of pro-inflammatory mediators is responsible for pain sensation along with paw swelling at site of

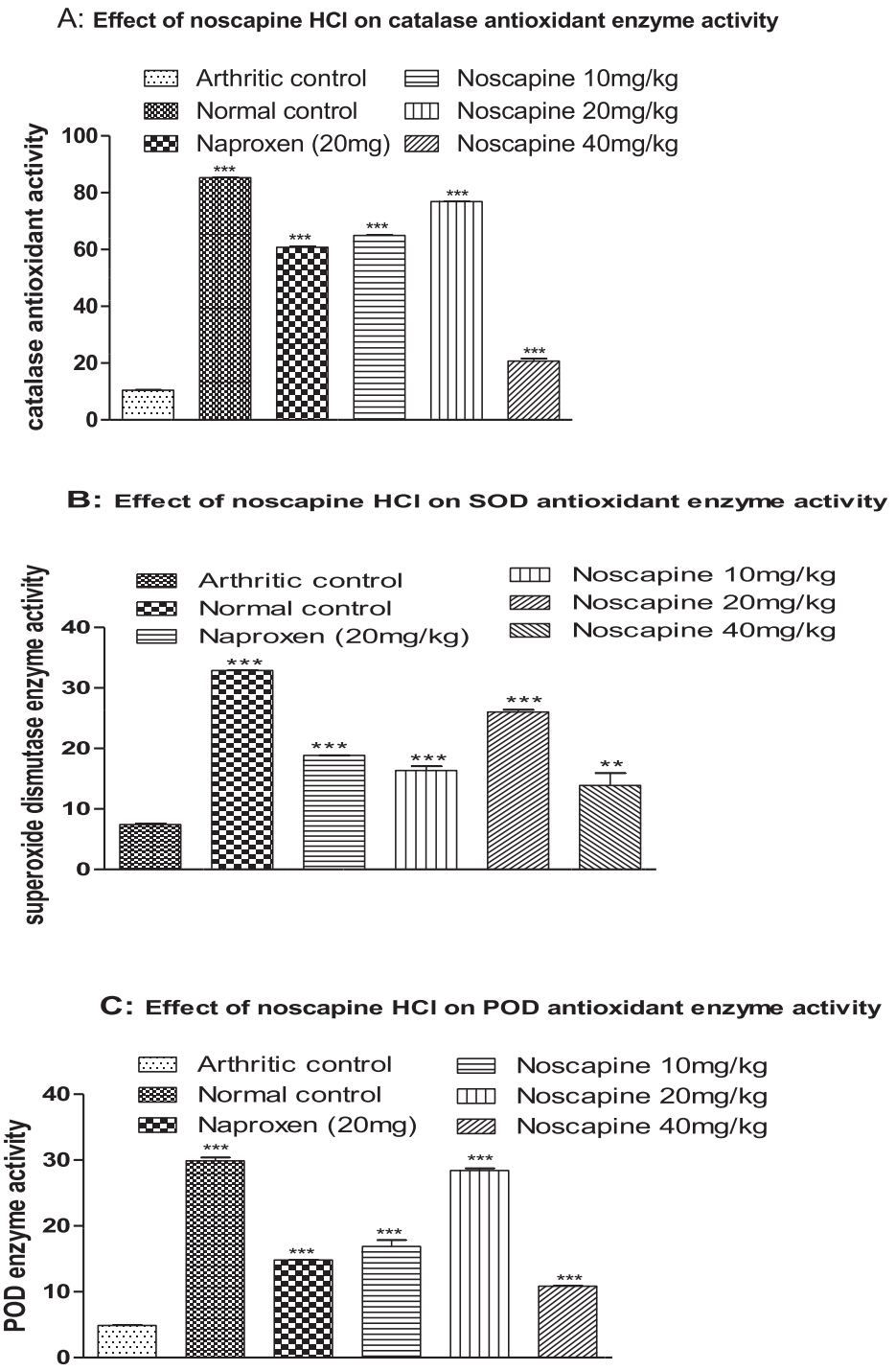

Fig. 7. Effect of noscapine hydrochloride at 10, 20 and 40 mg/kg orally for 28 days on antioxidant enzymes activity (CAT, SOD and POD) in CFA-induced arthritic model versus arthritic control.

injection, and also increased the disability of ankle joints. A significant upswing in paw swelling after subplantor shot of complete adjuvant is reflecting the grade of arthritis (Patil et al., 2012). Treatment with noscapine hydrochloride at all doses significantly reduced the paw volume and paw swelling via prevention of release of pro-inflammatory mediators.

In complete adjuvant induced arthritis, after immunization, arthritic score is an index of inflammation of joints. A selective inhibition in arthritic scoring supports its anti-inflammatory effect as shown in Fig. 3. Rheumatic cachexia is a general representative of arthritis which is presumed to be due to poor food intake and metabolism that are affected by inflammation and they are controlled by leptin (cytokine like hormone). It has been already reported that proliferation of T cells to promote the Th1 response in autoimmunity are stimulated by leptin. After adjuvant administrations increased concentrations of leptin associated with anorexia and cachexia (Patil et al., 2012). The discoveries of present study prove that administration of noscapine hydrochloride significantly attenuated the body weight loss in adjuvant induced arthritis might associated with prevention of inflammation and

hyperalgia along with improving the absorbance capacity of intestine by noscapine.

It has been described that reduction in level of RBCs and Hb associated with anemia produced due to dwindled reaction of erythropoietin of bone-marrow and annihilation of premature RBCs in complete adjuvant induced arthritis. ESR is an index of deferral reliability of RBCs in plasma. The number of RBCs is related with ESR. It also elaborates the establishment of endogenous proteins including alpha and beta globulins and fibrinogens. Augmented level of ESR is associated with stress, necrosis and inflammation. In current investigation, administration of noscapine hydrochloride significantly restores the level of Hb along with RBCs and down regulates the effect of ESR attributing its anti-inflammatory prospective (Patil et al., 2012). In addition, WBC are the first responders of immune system.

The increase in WBC count presented the signs of inflammatory disorder in arthritic control animals and increased may be due to the increase in concentration of macrophages colony factors and granulocytes which is associated with elevated expression of IL-1β inflammatory reactions which ultimately elevates the level of WBCs (Patil et al., 2012). In addition, expression of IL-1β was prevented by oral administration of noscapine hydrochloride that ultimately normalized the WBCs and potentiates its anti-inflammatory effect as discussed in Fig. 6a. So decreased expression of IL-1β may decreased the WBCs level in complete adjuvant induced arthritis. Serum elevated level of AST and ALT was found to be associated with elevated bradykinin level caused by inflammatory response whereas increased level of ALP are linked with bone obliteration due to lysosomal integrity (Uttra et al., 2019). In the present investigation AST, ALT and ALP elevated levels were detected in untreated arthritic control animals. Nonetheless, treatment with noscapine hydrochloride significantly reversed ALP level that may be due to stability of lysomal enzymes, as also discussed in membrane stabilization assay and decreased the bone destruction as observed by radiographic examination in this study. Similarly, creatinine and urea level were declined in tested group due to hepatoprotective effect of noscapine. Thus, normalization of blood and serum parameters by noscapine hydrochloride suggesting its persuasive immunomodulatory and anti-arthritic potential.

Moreover, the mediators like IL-6, NF-kB, IL-1β and TNF-α are linked with induction of arthritis, especially with the attack of inflammatory cells on synovial membrane that causes the bone and joint damage, hyperplasia and pannus formation (Manan et al., 2020). The expression of these inflammatory biomarkers was also evaluated by using RT-qPCR. Noscapine hydrochloride noticeably reduced these biomarkers as equated to rheumatic control rats.

In detail, IL-1β activates the production of osteoclast and matrix metalloproteinase formation leading to bone damage. Stimulation and differentiation of osteoclast are associated with NF-kB which results in synovial apoptosis and bone loss. NF-kB stimulates the immune system via a transcription of pro-inflammatory cytokines (IL-6, TNF-α and IL-1β). Activation of NF-kB exacerbates the rheumatoid arthritis. However, expression of NF-kB was inhibited in treatment groups as compared to diseased group. So, inhibitors of NF-kB possess the therapeutic efficacy against arthritis and could be used in the treatment of rheumatism (Manan et al., 2020). In the current study, noscapine hydrochloride notably reduced the expression of NF-kB as compared to diseased control rheumatic rats. Inflammatory biomarkers activate the expression of COX-2 in synovial membrane. Noscapine hydrochloride significantly decreased the expression of COX-2 and inhibited the angiogenesis and synovitis hallmarks in the synovial membrane. Furthermore, an augmented level of pro-inflammatory cytokines also causes the increased manifestation of prostaglandin E_2, and induces the infiltration of immune cells and ultimately causes the inflammation in organs and peripheral tissues. These parameters aggravate the rheumatic disorder. Consequently, down-regulation of the manifestation of these parameters may be an effective way to treat rheumatism (Gou et al., 2018). In the present investigation, noscapine hydrochloride significantly down-regulates the level of PGE_2 in treated groups in contrast to non-treated rheumatic control groups.

Furthermore, IL-4 and IL-10 are immuno-modulatory and anti-inflammatory cytokines (Latif et al., 2021). IL-4 obstruct auto-immune reaction arbitrated by Th_1 and upholds the generation of Th_2 (Shabbir et al., 2014). Although, IL-10 not only prevents Th_1 generated cytokines and (IFN-γ, IL-1, TNF-α) but also averts the mRNA expression of IL-18 and inhibit the antigen presenting functions of cells, and protects the joint spaces (Li et al., 2013). The results of present analysis reported that treatment with noscapine hydrochloride significantly up-regulates the effect of IL-4 and IL-10, and down- regulates the level of IL-1β, TNF-α, COX-2, NF-kB and IL-6 as presented in Fig. 6. However the reduction in inflammatory cytokines specifically IL-1β, TNF-α and IL-6 at noscapine (40 mg/kg dose) was not as much as it was observed at lower doses. This may be due to narrow therapeutic index or range of drug. Normally, the increasing doses result in an increased reaction, but only within a relatively small dose range; subsequent dose increases outside of this range have few additional effects. Furthermore, it can be a result of pharmacokinetic and pharmacodynamic variations among the animals as well. Similarly, throughout the course of therapy, even the same animal can exhibit variations in medication reactions from dose to dose (Tamargo et al., 2015; Maxwell, 2018; Rocca and Patrono, 2005).These observations validate anti rheumatic effect of noscapine and signify its use for the management of rheumatism.

It is established that, therapeutic products having anti-rheumatic efficacy also possess the antioxidant potential, hence we evaluated the antioxidant enzyme activity (CAT, SOD and POD) of noscapine hydrochloride. In present study level of these antioxidant enzymes was decreased in rheumatic control diseased rats, whereas administration of noscapine hydrochloride increased the concentration of these aforementioned antioxidant enzymes in the serum that avert the tissues destruction by increasing the concentration of these endogenous enzymes in treatment groups. Catalase protects from tissue damage because by reducing the production of hydrogen peroxide in the synovial membrane. A previous investigation strengthens our observation that antioxidants enzymes such as CAT, SOD and peroxidase were reported to be notably decreased in rheumatoid arthritis in contrast to normal control animals. Complete adjuvant elevates the level of oxidative stress and inflammatory cytokines which provokes the immune cells to generate the cytokines and enzymes to aggravate RA (Manan et al., 2020). Thus, it can be proposed that diminishing oxidative stress by noscapine could be one of the main mechanisms for reducing the manifestation of cytokines involved in chronic inflammation.

5. Conclusions

In conclusion, present findings demonstrate that oral administration of noscapine hydrochloride (20 mg/kg) in rheumatic animals, noticeably reduced protein denaturation, stabilized the lysosomal membrane, decreased the paw volume in formaldehyde and complete adjuvant induced RA in rats. Additionally, complete adjuvant model, treatment with noscapine hydrochloride significantly reduced the arthritic scoring, normalized the loss of body weight, hematological and serum alterations. Treatments also prevent the mRNA expression of pro-inflammatory biomarkers,

inflammatory enzymes (COX-2), together with dropping the level of prostaglandins and NF-kβ. Moreover, noscapine increased the level of antioxidant enzymes like peroxidase, catalase and superoxide dismutase that could be credited to its anti-rheumatic and immunomodulatory properties of iso-quinolone alkaloids.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Akhter, S., Irfan, H.M., Ur Rahman, S., Ansari, M., Mustafa, Z., Latif, M.B., 2021. Toxicity studies and anti-arthritic effect of mandelic acid (2-hydroxy-2-phenyl acetic acid) using *in vitro* and *in vivo* techniques. Pak. J. Pharm. Sci. 34 (2), 2281–2287.

Akhter, S., Irfan, H.M., Jahan, S., Shahzad, M., Latif, M.B., 2022. Nerolidol: a potential approach in rheumatoid arthritis through reduction of TNF-α, IL-1β, IL-6, NF-kB, COX-2 and antioxidant effect in CFA-induced arthritic model. Inflammopharmacol 30, 537–548.

Anosike, C.A., Obidoa, O., Ezeanyika, L.U., 2012. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum).* DARU J. Pharm. Sci. 20 (1), 1–7.

Bag, A., Kumar Bhattacharyya, S., Kumar Pal, N., Ranjan Chattopadhyay, R., 2013. Anti-inflammatory, anti-lipid peroxidative, antioxidant and membrane stabilizing activities of hydroalcoholic extract of *Terminalia chebula* fruits. Pharm. Bio 51 (12), 1515–1520.

Bellingan, G., 1999. Inflammatory cell activation in sepsis. British Med. Bull. 55 (1), 12–29.

Cedergren, J., Forslund, T., Sundqvist, T., Skogh, T., 2007. Intracellular oxidative activation in synovial fluid neutrophils from patients with rheumatoid arthritis but not from other arthritis patients. J. Rheumatol. 34 (11), 2162–2170.

Chandra, K., Salman, A.S., Mohd, A., Sweety, R., Ali, K.N., 2015. Protection against FCA induced oxidative stress induced DNA damage as a model of arthritis and In vitro anti-arthritic potential of *costus speciosus* rhizome extract. Inter. J. Pharma Phyto Res. 7 (2), 383–389.

de Cássia Da Silveira e Sá, R., Andrade, L.N., De Sousa, D.P., 2015. Sesquiterpenes from essential oils and anti-inflammatory activity. Natural. Prod. Comm. 10 (10), 1767–1774.

Desai Nilesh, V., Patkar Atul, A., Shinde Shilpa, S., Arwade Aboli, S., 2012. Protective effect of aqueous extract of *Aegle marmelos* against formaldehyde induced arthritis in rats. Inter. Res. J. Pharm. Appl. Sci. 2 (4), 66–72.

Gou, K.J., Zeng, R., Ren, X.D., Dou, Q.L., Yang, Q.B., Dong, Y., Qu, Y., 2018. Anti-rheumatoid arthritis effects in adjuvant- induced arthritis in rats and molecular docking studies of *Polygonum orientale* L. extracts. Immunol. Lett. 201, 59–69.

Hasan, U.H., Uttra, A.M., Rasool, S., 2015. Evaluation of in vitro and in vivo anti-arthritic potential of *Berberis calliobotrys*. Bangladesh J. Pharmacol. 10 (4), 807–819.

Latif, M.B., Shukla, S., Estrada, P.M., Ribeiro, S.P., Sekaly, R.P., Sharma, A.A., 2021. Immune mechanisms in cancer patients that lead to poor outcomes of SARS-CoV-2 infection. Transl. Res. 241, 83–95.

Li, Y., Wang, S., Wang, Y., Zhou, C., Chen, G., Shen, W., Shen, X., 2013. Inhibitory effect of the antimalarial agent artesunate on collagen-induced arthritis in rats through nuclear factor kappa B and mitogen-activated protein kinase signaling pathway. Trans. Res. 161 (2), 89–98.

Manan, M., Saleem, U., Akash, M.S.H., Qasim, M., Hayat, M., Raza, Z., Ahmad, B., 2020. Antiarthritic Potential of Comprehensively Standardized Extract of *Alternanthera bettzickiana: In Vitro and In Vivo* Studies. ACS Omega 5 (31), 19478–19496.

Maxwell, S.R.J., 2018. Clinical Therapeutics and Good Prescribing. In: Davidson's Principles and Practice of Medicine. 23rd ed. Elsevier, pp. 13–36.

Patil, M.V.K., Kandhare, A.D., Bhise, S.D., 2012. Anti-arthritic and anti-inflammatory activity of *Xanthium srtumarium* L. ethanolic extract in Freund's complete adjuvant induced arthritis. Biomed. Agi. Pathol. 2 (1), 6–15.

Rindfleisch, A.J., Muller, D., 2005. Diagnosis and management of rheumatoid arthritis. Ameri. Family Phys. 72 (6), 1037–1047.

Rocca, T.O.B., Patrono, C., 2005. Determinants of the interindividual variability in response to antiplatelet drugs. J. Thromb Haemost 3 (8), 1597–1602.

Sarsour, E.H., Kumar, M.G., Chaudhuri, L., Kalen, A.L., Goswami, P.C., 2009. Redox control of the cell cycle in health and disease. Anti Red. Sig. 11 (12), 2985–3011.

Scott, D.L., 2010. National Data Bank for Rheumatic Diseases, and University of Kansas School of Medicine, Wichita, KS, USA Department of Rheumatology. Lancet, 1094–1108.

Shabbir, A., Shahzad, M., Ali, A., Zia-ur-Rehman, M., 2016. Discovery of new benzothiazine derivative as modulator of pro-and anti-inflammatory cytokines in rheumatoid arthritis. Inflamm 39 (6), 1918–1929.

Shabbir, A., Shahzad, M., Ali, A., Zia-ur-Rehman, M., 2014. Anti-arthritic activity of N′-[(2, 4-dihydroxyphenyl) methylidene]-2-(3, 4-dimethyl-5, 5-dioxidopyrazolo [4, 3-c][1, 2] benzothiazin-1 (4H)-yl) acetohydrazide. Euro J. Pharmacol. 738, 263–272.

Souto, A.L., Tavares, J.F., Da Silva, M.S., Diniz, M.D.F.F.M., Athayde-Filho, D., Filgueiras, P., Barbosa Filho, J.M., 2011. Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. Molec 16 (10), 8515–8534.

Sung, B., Ahn, K.S., Aggarwal, B.B., 2010. Noscapine, a benzylisoquinoline alkaloid, sensitizes leukemic cells to chemotherapeutic agents and cytokines by modulating the NF-κB signaling pathway. Can Res. 70 (8), 3259–3268.

Tamargo, J., Le Heuzey, J.Y., Mabo, P., 2015. Narrow therapeutic index drugs: a clinical pharmacological consideration to flecainide. Eur. J. Clin. Pharmacol. 71 (5), 549–567.

Uttra, A.M., Shahzad, M., Shabbir, A., Jahan, S., Bukhari, I.A., Assiri, A.M., 2019. Ribes orientale: A novel therapeutic approach targeting rheumatoid arthritis with reference to pro-inflammatory cytokines, inflammatory enzymes and anti-inflammatory cytokines. J. Ethnopharmacol. 237, 92–107.

Walz, D.T., DiMartino, M.J., Misher, A., 1971. Adjuvant-induced arthritis in rats. II. Drug effects on physiologic, biochemical and immunologic parameters. J. Pharmacol. Exp. Therap. 178 (1), 223–231.

Wang, X., He, X., Zhang, C.-F., Guo, C.-R., Wang, C.-Z., Yuan, C.-S., 2017. Anti-arthritic effect of berberine on adjuvant-induced rheumatoid arthritis in rats. Biomed. Pharmacother. 89, 887–893.