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Research Article

Reduced Ulcerogenic Potential and Antiarthritic Effect of Chitosan–Naproxen Sodium Complexes

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Abstract. The purpose of this research was to address the utility of naproxen sodium–chitosan spray-dried complexes for antiulcer and antiarthritic activities. The cold stress technique was used to examine the ulcerogenic potential of naproxen sodium (NPX) and spray-dried formulations in the different doses. The ulcerations reduced with the dose of spray-dried complexes of naproxen sodium and chitosan. The conspicuous hemorrhagic lesions were visible in the morphological features of the animal treated with naproxen 50 mg/kg (p.o.). Thus, the results suggest that the spray-dried naproxen sodium–chitosan complex (NPXF) was not corrosive to the gastric mucosa at high doses of 50, 100, and 200 mg/kg (p.o.) under stressful conditions. It is evident from the present investigation that NPXF does not possess any ulcerogenic potential in comparison to naproxen which, under stressful conditions, led to the hypersecretion of HCl, culminating to petichial hemorrhages in the gastric mucosa of the animals. The biphasic pattern was observed in the various arthritic parameters. The rise in paw volume, joint diameter, WBC count, arthritis score, and fall in body weight was significantly ameliorated in the animals treated with NPXF (5, 10, and 20 mg/kg, p.o). At the end of the study, slight erythema was visible in the naproxen-treated animals. However, no erythema, redness, or ulcers were visible in the animals treated with NPXF. Thus, the direct compression properties and reduced ulcerogenic activity, combined with the demonstrated solubilizing power and analgesic effect enhancer ability toward the drug, make naproxen sodium–chitosan spray-dried complexes particularly suitable for developing a reduced-dose, fast-release, solid oral dosage form of naproxen.

KEY WORDS: antiarthritic; chitosan complexes; ulcer.

INTRODUCTION

Disorders that affect the joints and their components—muscles, bones, cartilage, and tendons—are considered connective tissue diseases because these structures contain large amounts of connective tissues that can become inflamed during different autoimmune disorders like rheumatoid arthritis. The drugs that reduce inflammation include non-steroidal anti-inflammatory drugs (NSAIDs). However, peptic ulceration is their major unavoidable side effect (1). Naproxen sodium (NPX), a non-steroidal anti-inflammatory, weakly acidic, crystalline drug is used to relieve the minor pains of arthritis. However, it exhibits gastric toxicities, mucosal ulcerations, and hemorrhage due to inhibition of prostaglandin production.

The severity of these side effects can be reduced by lowering the peak plasma concentrations and sustaining the drug action (2–5). The use of polyelectrolytes in the design of controlled-release drug formulations has received increasing attention in recent years. As excipients, polyelectrolytes have shown to affect the release of oppositely charged drugs due to the formation of stable ionic complexes. Oppositely charged polyelectrolytes such as sodium alginate–chitosan, polyacrylic acid–chitosan, and chitosan–carrageenan have been utilized in the design of controlled-release formulations (6–11). Chitosan (CH) is a linear cationic polyelectrolyte in which the degree of ionization of the amine groups depends significantly on the pH of the media. At acidic pH, these groups are protonated, acquire positive charge, and coagulate upon the addition of negatively charged drugs (5,12–15). Previous research from our laboratory has provided an evidence to confirm the *in situ* complexation in the matrices containing CH, κ-carrageenan

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ABBREVIATIONS: κ-Ca, κ Carrageenan; CH, Chitosan; FCA, Freund's complete adjuvant; HPMC, Hydroxypropyl methyl cellulose; NPX, Naproxen sodium; NSAIDs, Non-steroidal anti-inflammatory drugs; NPXF, Spray-dried naproxen sodium–chitosan complex; SD, Spray drying; USP, United State Pharmacopoeia; veh, Vehicle; WC, Water column.

(κ-Ca), and NPX (11). The possibility of ionic interactions in CH and NPX complexes prepared by different drying techniques was further explored, and the characterization of ionic complexes, the evidence of charge presentation in the swollen gel matrices, and their dissolution profiles in the different media have been addressed (16). The influence of oppositely charged polymers and dissolution media on the rheology of hydrated matrices containing spray-dried NPX–CH complex (NPXF) has been addressed in our recent work (17). Taking the advantage of the antiulcer property of CH that has been reported in the literature (5), an attempt has been made to investigate the antiulcer and antiarthritic potential of NPXF in the present manuscript.

MATERIALS AND METHODS

Materials

CH (87% deacetylation), with molecular weight of 80 kDa, was used (Marine Chemicals, Chennai, India); and NPX were obtained as gift samples from Divi Laboratories Pvt. Ltd., Hyderabad, India. All other chemicals were purchased and were of analytical grade.

Methods

Preparation of NPXF by the Spray Drying Technique

Based on the average maximum binding capacity between CH and NPX (10), 3 g CH was dissolved in 100 ml of 1% (*v*/*v*) acetic acid solution and was stirred continuously for 24 h at 50 rpm (Whirlmatic Motorless Magnetic Stirrer, model WS-MEGA; Spectra Lab, Mumbai, India) to ensure maximum swelling of CH. An equal amount of NPX was mixed separately in 100 ml deionized water. Both solutions were mixed together and were stirred on a six-station magnetic stirrer at 50 rpm for 24 h at room temperature. The resultant dispersion of NPX and CH in a 1:1 ratio was then spray-dried in a co-current spray system (Twin Cyclon Lab Spray Drier, LU-222 Advanced Model, Labultima, Mumbai) with a nozzle size of 0.7 mm, two-fluid spray nozzles at inlet temperatures of 165–170°C, and an outlet temperature of 108–115°C. The vacuum obtained at 45% aspirator was −110 mm WC. The sample was pumped through 0.25 cm annular air orifice at a rate 2 ml/min (approximately 14.5%) with atomization air pressure of 2 kg/cm^2. NPXF sample was collected and stored in the desiccator.

Pharmacological Study

The animal experiments were conducted in full compliance with local, national, ethical, and regulatory principles and local licensing regulations, per the spirit of the Association for Assessment and Accreditation of Laboratory Animal Care. The protocol approval number provided by the Institutional Animal Ethical Committee (IAEC) was CPCSEA/04/2010, with IAEC registration number 100/1999/CPCSEA. The 250-mg NPXF sample that contained a drug equivalent to 125 mg of NPX was evaluated for ulcerogenic and antiarthritic studies, respectively, as per the protocols described below.

Ulcerogenic Study. Samples of NPX and NPXF were evaluated for ulcerogenic activity using Rainsford's cold stress model (18) in which accurately weighed 50, 100, and 200 mg of NPXF samples were dispersed separately in 1 ml of 1% sodium carboxymethyl cellulose (CMC) vehicle. A standard sample of pure drug was prepared by dissolving accurately weighed 50 mg NPX in 1 ml of 1% sodium CMC vehicle. The samples were administered orally in healthy female Wistar rats weighing 200–250 g that were divided into five groups of six animals in each group as follows:

Group 1: Control group—vehicle (Veh) 1 ml of 1% sodium CMC per day
Group 2: NPX 50 mg/day in 1 ml of 1% sodium CMC
Group 3: NPXF 50 mg/day equivalent to 25 mg NPX in 1 ml of 1% sodium CMC
Group 4: NPXF 100 mg/day equivalent to 50 mg NPX in 1 ml of 1% sodium CMC
Group 5: NPXF 200 mg/day equivalent to 100 mg NPX in 1 ml of 1% sodium CMC

Thereafter, the animals were put under stressful conditions in separate polypropylene cages at a temperature of −15°C for a period of 15 min. After the exposure to stress, the animals were killed by cervical dislocation. The stomach was dissected out and cut along the greater curvature. The mucosal surface was washed with saline and an image of the stomach captured using a CCD scanner at a resolution of 2,400 DPI. The ulcerogenic response was calculated using the method of Cioli *et al.* (19) and the ulcer index determined. The ulcer area was measured and scored according to Table I. All data were analyzed by two-way ANOVA followed by Bonferroni posttest (*$p<0.05$, **$p<0.01$, ***$p<0.001$). Ulcer area was calculated using Adobe Photoshop and Image J software.

Antiarthritic Study. Arthritis was induced by a single intradermal injection of Freund's complete adjuvant (FCA) containing 1.0 mg dry heat-killed *Mycobacterium tuberculae* per milliliter sterile paraffin oil into the subplantar region of the foot pad of the left hind paw of healthy female Wistar rats weighing 200–250 g. One-milliliter tuberculine syringe with 26-gauge needle was used to administer Freund's complete adjuvant. The rats were anesthetized with light ether inhalation prior to and during adjuvant injection. For antiarthritic study, accurately weighed NPX and NPXF samples indicated in groups 1 to 5 were dispersed separately in 1 ml of 1% sodium CMC

Table I. Scoring of Ulcer Area

Sr. no.	Ulcerogenic response	Score
1	Ulcers <1 mm	1
2	Ulcers <1–2 mm	2
3	Ulcers <2–3 mm	3
4	Ulcers <3–4 mm	4
5	Ulcers <4–5 mm	5
6	Ulcers >5 mm	10
7	Perforated lesions	25

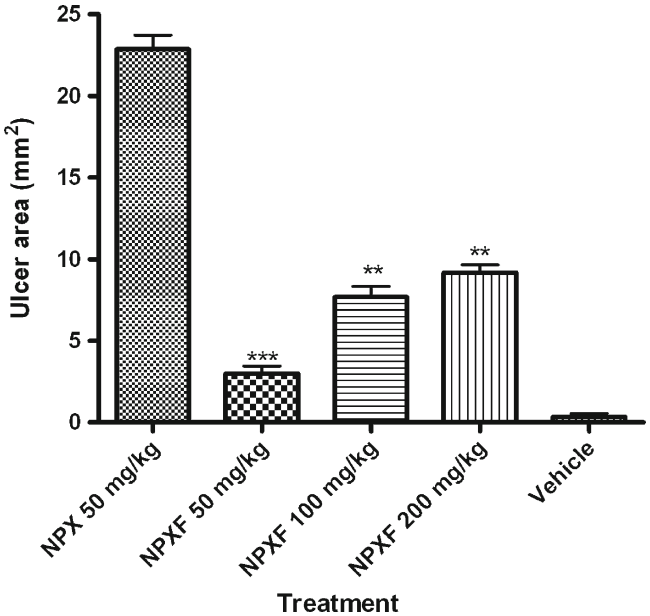

Fig. 1. Ulcer area of NPX, NPXF (50 mg/kg), NPXF (100 mg/kg), NPXF (200 mg/kg), and vehicle

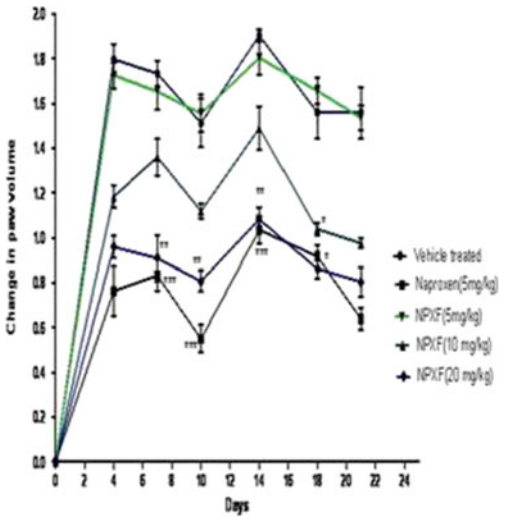

Fig. 3. Effect of NPX, NPXF, and vehicle on paw volume at the doses of 5, 10, and 20 mg/kg, p.o.

vehicle and wereadministered orally for 21 days to the experimental animals divided into the following five groups of six animals in each group.

Group 1: Control group—vehicle (Veh) 1 ml of 1% sodium CMC per day
Group 2: NPX 5 mg kg^{-1} day^{-1} in 1 ml of 1% sodium CMC
Group3: NPXF 5 mg kg^{-1} day^{-1} equivalent to 2.5 mg NPX in 1 ml of 1% sodium CMC
Group 4: NPXF 10 mg kg^{-1} day^{-1} equivalent to 5 mg NPX in 1 ml of 1% sodium CMC
Group5: NPXF 20 mg/day equivalent to 10 mg NPX in 1 ml of 1% sodium CMC

The progression of FCA-induced arthritis was evaluated by measuring the following parameters on 0, 4, 7, 10, 13, 14, 17, 19, and 21 days after adjuvant injection. All data was analyzed by two-way ANOVA followed by Bonferroni posttest (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Paw Volume. The swelling in the hind paw from the ankle was measured periodically on the days mentioned above using plethysmometer (Ugo Basile, Italy) (20).

Arthritis Score. Rats were scored for arthritis (arthritis index) daily by a set visual criterion (21).

The following scoring system was used:

Normal paw = 0
Swelling and erythema of the digits = 2
Mild swelling and erythema of the digits = 3
Gross deformity and inability to use the limb = 4

Body Weight. The body weight of all the animals was recorded using electronic balance (22).

WBC Count. The total WBC count was measured using Neubar's chamber as an indication of the inflammatory response (23).

Joint Diameter. The joint diameter was measured in millimeters with the help of Vernier calipers, and change in joint diameter was calculated (21,22).

RESULTS AND DISCUSSION

It is known that NPX is a powerful NSAID used in a plethora of inflammatory diseases. However, its ulcerogenic activity has markedly limited its use in the geriatric patients. CH exhibits anti-cholesterolemic, antiulcer, and anti-uricemic properties when orally administered and is potentially suitable

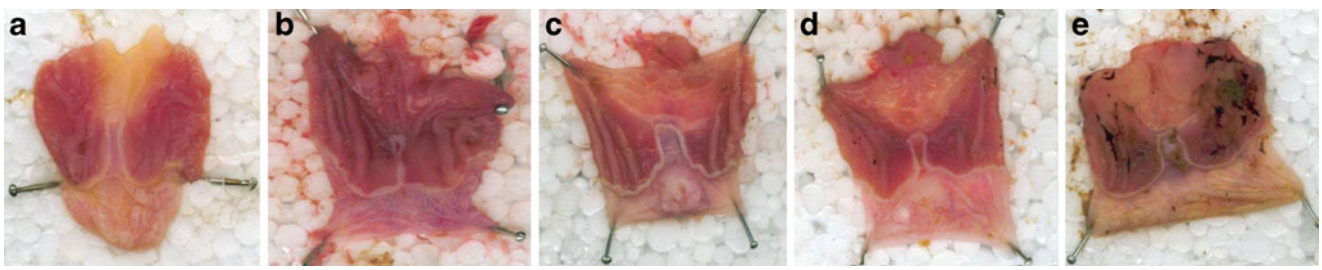

Fig. 2. Morphological features of healthy animal (**a**); of animals treated with NPXF 50 mg/kg (**b**), NPXF 100 mg/kg (**c**), and NPXF (200 mg/kg) (**d**); and of an animal treated with NPX (**e**)

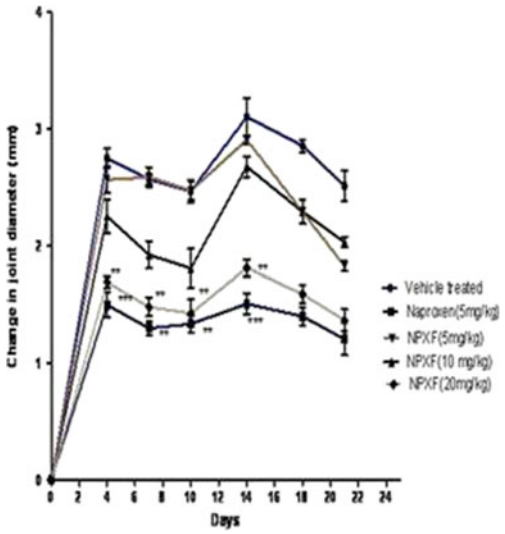

Fig. 4. Effect of NPX, NPXF, and vehicle on joint diameter at the doses of 5, 10, and 20 mg/kg, p.o.

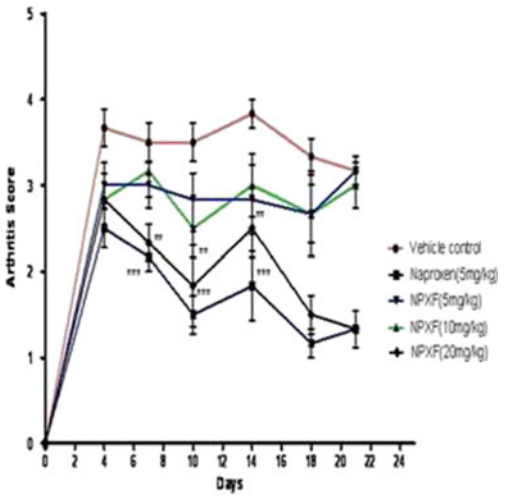

Fig. 6. Effect of NPX, NPXF, and vehicle on paw arthritis score at the doses of 5, 10, and 20 mg/kg, p.o.

for the prevention of coeliac disease (5,24,25). This set of properties stems from the capacity to bind specifically fatty acids, bile acids, phospholipids, uric acid, and the toxic gliadin fraction. The scope of the present study was to investigate whether an ulcerogenic potential of NPX can be reduced by its ionic complexation with CH.

Ulcerogenic Study

Cold stress technique was used to examine the ulcerogenic potential of NPX and NPXF in doses of 50, 100, and 200 mg/kg, p.o., respectively.

In Rainsford's cold stress model, NPXF was administered orally ten times in therapeutic dose per kilogram body weight of healthy female Wistar rats of 200–250 g weight to assess ulcerogenic potential at high doses. Figure 1 demonstrates the ulcer area (in square millimeters) in the five groups of animals. All data were analyzed by two-way ANOVA followed by Bonferroni posttest (*$p<0.05$, **$p<0.01$, ***$p<0.001$). The ulcer area seemed to be increasing with NPXF dose, as indicated in Fig. 1. It is noteworthy that the ulcer area of NPXF 100 mg was lesser than that of NPX 50 mg, supporting the reduction in an ulcerogenic potential of the latter probably due to the polyelectrolyte complexation between CH and the drug. Figure 2a shows the morphological features of the healthy animal. No ulcers were visible in the glandular portion of the stomach. Figure 2b–e presents the morphological features of an animal treated with NPXF at doses of 50, 100, and 200 mg/kg, p.o., respectively. As indicated in Fig. 2e, the conspicuous hemorrhagic lesions were visible in the morphological features of animals treated with NPX 50 mg/kg (p.o.). It is evident that NPXF possesses less ulcerogenic potential in comparison to NPX, which under stressful conditions led to the hypersecretion of HCl,

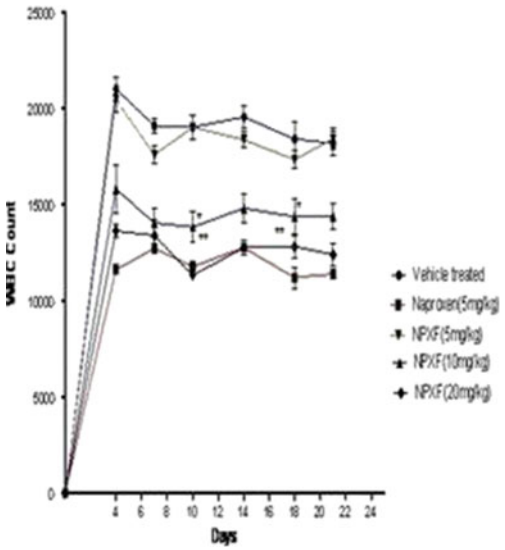

Fig. 5. Effect of NPX, NPXF, and vehicle WBC count at the doses of 5, 10, and 20 mg/kg, p.o.

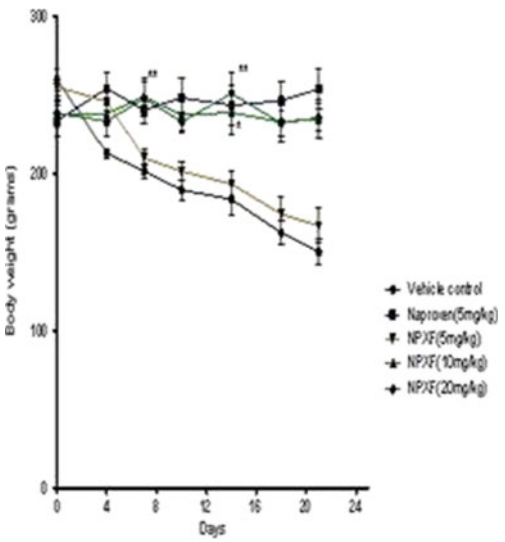

Fig. 7. Effect of naproxen, naproxen formulations, and vehicle on body weight at the doses of 5, 10, and 20 mg/kg, p.o.

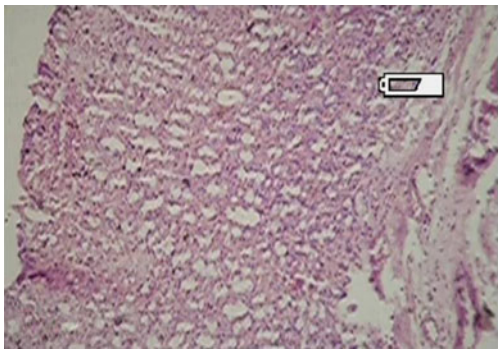

Fig. 8. Histopathological image of the stomach of rat treated by NPXF showing minimal inflammation. Hematoxylin–eosin stain was used and the image captured at ×40 magnification

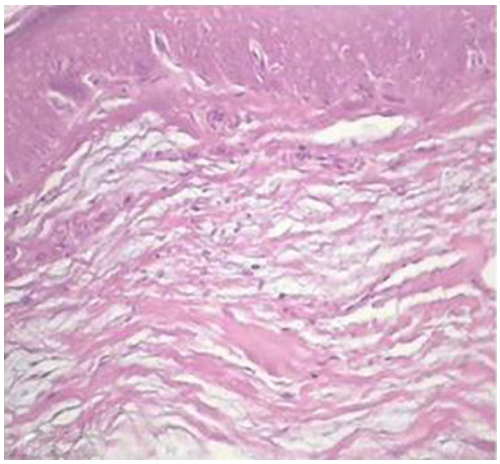

Fig. 10. Histopathological image of paw treated with NPX showing no inflammation. Hematoxylin–eosin stain was used and the image captured at ×40 magnification

culminating to petichial hemorrhages in the gastric mucosa of the animals. The results suggest that NPXF at high doses of 50, 100, and 200 mg/kg (p.o.) under stressful conditions was less corrosive to the gastric mucosa than NPX 50 mg. The antiulcer activity of CH cannot be neglected in the overall antiulcer activity of NPXF.

Antiarthritic Study

In the antiarthritic study of NPXF, various parameters like paw volume, joint diameter, WBC count, and arthritis score were monitored throughout the dosage regimen. All data were analyzed by two-way ANOVA followed by Bonferroni posttest (*$p<0.05$, **$p<0.01$, ***$p<0.001$). Figure 3 shows the effect of NPXF on paw volume at doses of 5, 10, and 20 mg/kg (p.o.). Figure 4 indicates the effect of NPXF on joint diameter at doses of 5, 10, and 20 mg/kg (p.o.). A biphasic response was observed in various arthritis parameters like paw volume, WBC count, joint diameter, *etc.*, that exhibited a rise in values until day 4 followed by a reduction on day 10 and attained maxima on the 14th day during the period of pharmacological evaluation. There was a biphasic response to the change in joint diameter and the elevation in the joint diameter that was inhibited by NPXF at the dose of 20 mg/kg (p.o.) throughout the treatment period ($p<0.01$). An effect of NPXF on WBC count at doses of 5, 10, and 20 mg/kg (p.o.) has been depicted in Fig. 5. The total WBC count was elevated in the vehicle-treated group and the animals treated with NPXF (5 mg/kg, p.o) equivalent to 2.5 mg/kg (p.o.) NPX. This rise was significantly inhibited in the animals treated with NPX 5 mg and NPXF 10 and 20 mg/kg (p.o.), respectively, due to an increase in the concentration of NPX with the dose of NPXF. NPXF 5 mg/kg did not behave differently from the blank vehicle due to the insignificant concentration of NPX in it.

Figure 6 presents an effect of NPXF on paw arthritis score at doses of 5, 10, and 20 mg/kg (p.o.). An arthritis score of 4 was observed in the animals in the vehicle-treated group. Figure 7 demonstrates the effect of naproxen formulations on body weight at the doses of 5, 10, and 20 mg/kg (p.o.). Due to the absence of antiarthritic activity, body weight loss was observed in the animals treated with vehicle and NPXF (5 mg/kg, p.o.), whereas the animal groups treated with higher doses of NPXF exhibited no significant loss of weight due to their antiarthritic activity. NPX at a dose of 5 mg/kg (p.o.) and NPXF at a dose of 20 mg/kg (p.o.) exhibited significant

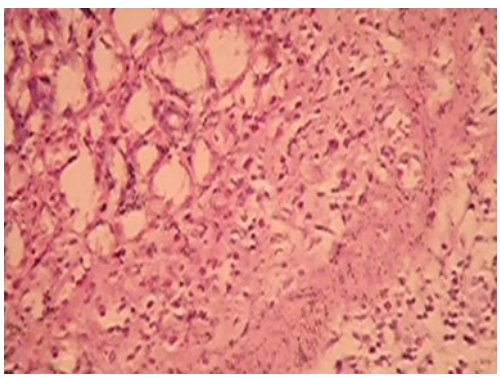

Fig. 9. Histopathological image of the stomach treated with NPX showing severe inflammation. Hematoxylin–eosin stain was used and the image captured at ×40 magnification

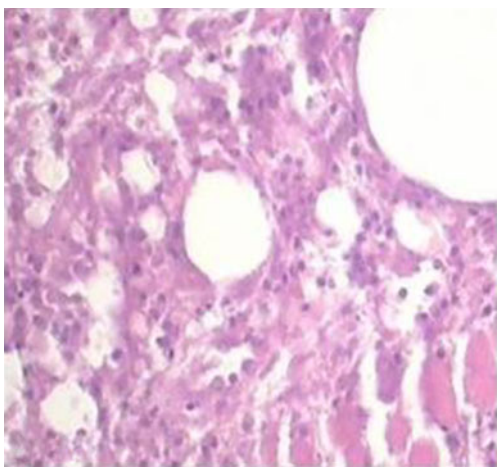

Fig. 11. Histopathological image of paw treated with NPXF 5 mg/kg (p.o.) showing slight inflammation. Hematoxylin–eosin stain was used and the image captured at ×40 magnification

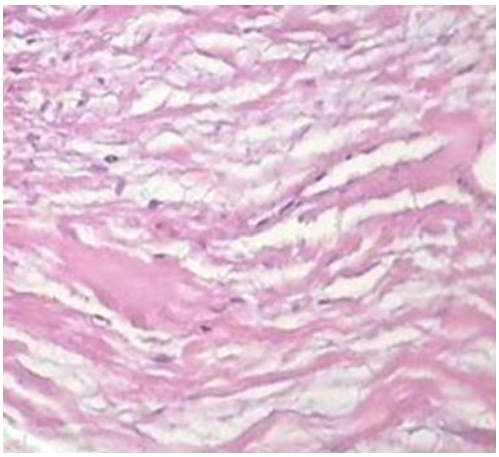

Fig. 12. Histopathological image of paw treated with NPXF 10 mg/kg, p.o (no inflammation). Hematoxylin–eosin stain was used and the image captured at ×40 magnification

amelioration of the disease. The rise in paw volume, joint diameter, WBC count, arthritis score, and fall in body weight was significantly ameliorated in the animals ($p<0.01$; Figs. 4, 5, 6, and 7). At the end of the study, slight erythema was visible in the NPX-treated animals, which was absent in those treated with NPXF. The histopathological studies were in accordance with the above observations. Inflammation was seen in the gastric tissues of the stomach of the animals, whereas no damage was observed in the animals treated with all the three doses of NPXF (Figs. 8 and 9). The histopathological analysis of the paw of the control animals exhibited prominent lesions, whereas the animals in the NPX- and NPXF-treated groups presented no lesions and inflammation (Figs. 10, 11, 12, 13, and 14). Thus, the results support that the ulcerogenic potential of NPX can be reduced by virtue of polyelectrolyte complexation using the spray drying technique. The investigation gives pharmacological credence to an important facet of polyelectrolyte complex formulation.

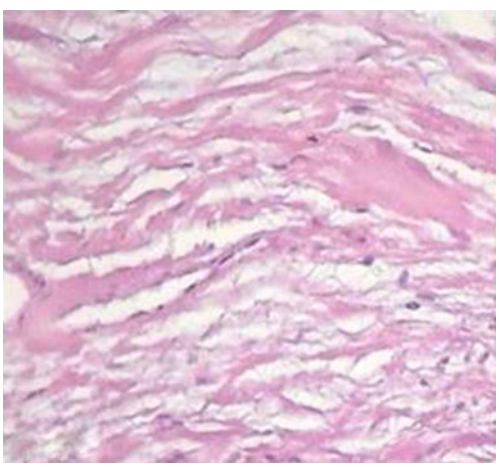

Fig. 13. Histopathological image of paw treated with NPXF 20 mg/kg (p.o.) showing no inflammation. Hematoxyline–eosin stain was used and the image captured at ×40 magnification

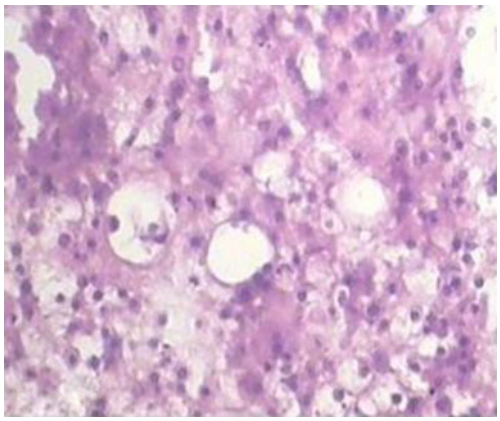

Fig. 14. Histopathological image of arthritic paw showing severe inflammation. Hematoxyline–eosin stain was used and the image captured at ×40 magnification

CONCLUSION

It could be concluded from the present investigation that the spray-dried polyelectrolyte complexes have reduced ulcerogenecity and adequate therapeutic potential, which will open novel vistas in the field of NSAID therapy and pave the way for better management of pain in patients.

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