



Regular Articles

## Evaluation of the analgesic and anti-inflammatory activity of *Moringa concanensis* tender fruits

Ch. V. Rao \*, Md. Talib Hussain, Arti R. Verma, Nishant Kumar,  
M. Vijayakumar, G.D. Reddy

Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (CSIR),  
Rana Pratap Marg, Post Box No. 436, Lucknow-226 001, Uttar Pradesh, India

### Abstract

The analgesic and anti-inflammatory effects of an ethanolic extract of *Moringa concanensis* tender pod-like fruits in experimental animals were evaluated. The ethanolic extract of *M. concanensis* at the dose levels of 200 and 400 mg/kg, was administered orally, once daily for 3 days for evaluation of the analgesic (analgesy-meter-induced pain, acetic acid-induced writhing and reaction time in the hot plate test) and anti-inflammatory (carrageenan-induced paw edema) effects. The activities of lysosomal enzymes and glycoproteins were investigated in fundic stomach and liver homogenate of rats with adjuvant-induced arthritis. A significant analgesic activity of *M. concanensis* was observed in mice; 22.53 % and 51.47 % ( $P<0.05$  and  $P<0.001$ ) protection against mechanical pain, 22.73 % and 51.63 % ( $P<0.05$ ), protection against acetic acid-induced writhing and 62.20 % and 125.59 % ( $P<0.05$  and  $P<0.01$ ) protection against thermal-induced pain. Aspirin and pentazocine potentiated the analgesic effect of *M. Concanensis*. However, *M. concanensis* caused a significant ( $P<0.05$  and  $P<0.001$ ) and dose dependent inhibition of paw swelling caused by the carrageenan after 3 hr equivalent to 26.28 % and 44.23 % protection. Under the same experimental conditions, nimuslide (50 mg/kg; p.o.) potentiates the anti-inflammatory activity of *M. concanensis*. Oral administration of *M. concanensis* showed a tendency to reduce the elevated levels of the lysosomal enzymes (acid phosphatase and N-acetyl glucosaminidase) significantly and they reverted to near normal values, which may be due to stabilization of the lysosomal membrane. The glycoprotein (total hexose and sialic acid) contents were increased following treatment of *M. concanensis* in liver and stomach homogenate of rats with adjuvant-induced arthritis. Our results show that *Moringa concanensis* possess significant analgesic and anti-inflammatory activity.

**Key words:** *Moringa concanensis*; pain; inflammation

### Introduction

The family moringaceae has a single genus *Moringa*, with 13 species, of which only 2 species have been recorded in India, *Moringa concanensis* and

*Moringa oleifera*. *M. concanensis* Nimmo is a small tree indigenous to Northwest India resembling *M. oleifera* and it is abundant in Rajasthan, the dry hills of Konkan, Andhra Pradesh and is commonly found on recent alluvial land in or near the sandy beds of rivers and streams<sup>[1]</sup>. The tree is valued mainly for its tender pods, which are valued as a vegetable. The tender pod-like fruits of *Moringa* are cut into slices and used in culinary preparations; they are also used in the preparation of various types of vegetable curries and pickles. The Indian traditional systems of medicine,

\* Author to whom correspondence should be addressed. Address: Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (CSIR), Rana Pratap Marg, Post Box No. 436, Lucknow-226 001, Uttar Pradesh, India; Phone: +91-522-2205831-35 Ext: 352; E-mail: ethnopharmacology1@yahoo.co.in



especially Ayurveda, have put forward a number of therapeutic claims for these plant drugs. The whole plant parts of the tree are used in the treatment of ascites, rheumatism, venomous bites and painful swellings [2]. The seeds of *M. concanensis* contain 30.07 %, 6.00 %, 5.88 % and 9.00 % of protein, fiber, moisture and ash, respectively. The oil was found to contain high levels of oleic acid (up to 68.00 %) followed by palmitic, arachidic, stearic, and behenic acids up to levels of 11.04 %, 7.09 %, 3.58 %, and 3.44 %, respectively [3]. To the best of our knowledge no scientific report has described traditional claims regarding *M. concanensis*. In continuation of our studies on the biological evaluation of various plant drugs in our laboratory, we thought it important to verify the claims made for *M. concanensis* with regard to anti-inflammatory and analgesic activities in experimental animals.

## Materials and methods

### Plant material

The tender pod-like fruits of the cultivated variety of *M. concanensis* were collected in the month of February and the herbarium sheet was identified, authenticated taxonomically and confirmed by Dr. Kaushal Kumar, Scientist, Ethnobotany and Taxonomy, National Botanical Research Institute, Lucknow, India. A voucher specimen (NAB 360242) of the collected sample was deposited in the departmental museum for future reference.

### Animals

Male Sprague-Dawley rats (150-175 g) and albino mice (25-30 g) were obtained from the animal colony of the National Laboratory Animal Centre, Lucknow, India. They were randomly distributed into groups and housed in cages (6 per cage) and maintained under standard conditions at 26±2 °C and relative humidity 44 %-56 % and 10 h light: 14 h dark cycles each day for one week before and during the experiments.

All animals were fed the standard rodent pellet diet (Dayal, India) and drank water ad libitum. All studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India.

### Preparation of extracts

The fresh fruits of *M. concanensis* were washed with running tap water and shade dried. The dried materials were powdered and passed through a 100-mesh sieve. The coarsely powdered material was extracted three times with ethanol (80 % v/v) and filtered. The filtrate was concentrated at 40±2 °C on a rotary evaporator (Buchi, USA) and then freeze-dried (Freezone® 4.5, Labconco, USA) at high vacuum ( $133 \times 10^{-3}$  mBar) and low temperature -40 °C±2 °C to obtain a dry residue (yield 3.4 %, w/w).

### Preliminary phytochemical screening and HPTLC analysis

Preliminary qualitative phytochemical screening of *M. concanensis* gave a positive result for steroids, alkaloids, terpenoids, phenolics, flavanoids, and tannins. High performance thin layer chromatography (HPTLC) studies of the ethanolic extract of *M. concanensis* were carried out on pre-activated (100 °C) silica gel plates (Merck 60 F254) as the stationary phase and toluene : ethyl acetate : formic acid (7:3:1) as the mobile phase. The extract was spotted using a Camag Linomat IV spotter. After development, the plates were dried, observed at UV 366 and densitometrically scanned on a TLC scanner III using CAT software at a wavelength 366 nm (CAMAG, Switzerland).

### Drug treatment

The ethanolic extract of *M. concanensis* (suspended in 1 % carboxy methyl cellulose) at doses of 200 and 400 mg/kg body wt. was administered once daily for three consecutive days. Nimusilide (Cipla, India) at a dose of 50 mg/kg, p.o. was used as



the standard anti-inflammatory agent, and pentazocine (Ranbaxy, India) 10 mg/kg, i.p. and aspirin (Astra-IDL Ltd, India) 25 mg/kg, i.p. were used as standard analgesic agents. A control group of animals received suspension of 1 % carboxy methylcellulose in distilled water. Experiments were conducted on day 3, 60 min for anti-inflammatory activity and 30 min for analgesic activity after the last drug or vehicle administration [4].

### ***Analgesy-meter induced pain***

The analgesic effect of *M. concanensis* was tested in mice of either sex using an Ugo Basile 37215 analgesy-meter. This method involves the application of force to the paw of the mice using the analgesy-meter, which exerts a force that increases at a constant rate. The mice were gently placed between the plinth and plunger. The instrument was switched on and a constant motor rate was used to drive the plunger on to the paw of the mice. When the mice struggle the instrument is switched off and the force at which the animals felt pain is read on a scale calibrated in gram  $\times 10$  by a pointer [5].

### ***Acetic acid- induced writhing***

Acetic acid solution at a dose of 10 ml/kg (0.6 %) was injected i.p. and the number of writhing motions during the following 15 minute period was observed [6]. Significant reductions in the number of writhing motions following drug treatment as compared with vehicle treatment were considered as a positive analgesic response. The percentage inhibition of writhing was calculated as follows:

$$\% \text{ Inhibition} = (1 - VT / VC) \times 100$$

VT = number of writhing motions in drug-treated mice.

VC = number of writhing motions in the control group of mice

### ***Hot plate reaction time in mice***

Mice were screened by placing them on a hot plate maintained at  $55 \pm 1$  °C and recording the reaction time in seconds for paw licking or jumping. Only

mice which reacted within fifteen seconds and which did not show large variation when tested on four separate occasions, each fifteen minutes apart, were used for the test. Pentazocine (10 mg/kg, i.p.) was used as reference standard. The time for paw licking or jumping on the heated plate of the analgesia-meter was taken as the reaction time [7].

### ***Carrageenan- induced paw edema***

Rats were injected with 0.1 ml 1 % carrageenan into the subplantar region of the left hind paw [8]. The paw was marked with ink at the level of the lateral malleolus and dipped in a perspex cell up to this mark. The paw volume was measured by displacement of the water column in a plethysmometer (Ugo Basile, Italy) immediately after carrageenan application (time zero) and 3 h after the stimulus. A reduction in the paw volume compared with the vehicle-treated control animals was considered as an anti-inflammatory response.

### ***Adjuvant- induced arthritis***

Arthritis was induced by the intradermal injection of 0.1 ml complete Freund's adjuvant (CFA) in the right hind paw [9]. The adjuvant contained 10 mg heat killed *mycobacterium tuberculosis* in 1 ml paraffin oil. *M. concanensis* was administered orally at a dose of 400 mg/kg in 1 % CMC for 8 days and from the 11 to 18<sup>th</sup> day in post CFA rats. At the end of the 18<sup>th</sup> day the rats were sacrificed and the stomach and liver were carefully removed, and rinsed in chilled 0.15 M Tris KCl buffer (pH 7.4). The stomach was slit opened longitudinally, cleaned and flushed with the buffer 5 - 6 times. The fundic part of the stomach and the liver were then blotted dry, weighed and homogenized with a Potter- Elvehjem glass homogenizer in 0.15 M Tris KCl buffer (pH 7.4) to yield a 10 % (w/v) homogenate.

### ***Assay of lysosomal enzymes and glycoprotein content***

The activities of lysosomal enzymes were

investigated in fundic stomach and liver homogenate. Acid phosphatase was assayed by the method of King [10] using disodium phenyl phosphate as a substrate and is expressed as  $\mu\text{moles} \times 10^{-2}$  phenol. Macromolecules such as total hexose [11] and sialic acid [12] were estimated and expressed as  $\mu\text{g}/100$  mg wet tissue. N-acetyl glucosaminidase was determined by the method of Marhun [13] and expressed as  $\mu\text{moles} \times 10^{-2}$  *p*-nitro phenol liberated/h/mg protein.

### Statistical analysis

All the data are presented as mean  $\pm$  S.E.M and one-way analysis of variance (ANOVA) and Newman-Keuls Multiple Comparison Test were applied for determining the statistical significance between different groups.

### Results and discussion

The preliminary HPTLC studies revealed that the solvent system toluene: ethyl acetate : formic acid (7:3:1) was ideal and gave well-resolved sample peaks (Fig. 1). The spots of the chromatogram were

visualized at 366 nm with a 400 k filter at  $R_f$  values of 0.04, 0.44, 0.53, 0.61, 0.69, 0.72, 0.81, 0.87 and 0.96. The densitometric scanning at 366 nm gave five major spots with an area of 13.52, 14.29, 12.67, 10.45, 15.85 % at  $R_f$  values of 0.53, 0.61, 0.69, 0.72 and 0.96, respectively.

*M. concanensis* improved the pain threshold of analgesy-meter induced pain. The ethanolic extract of *M. concanensis* at dose levels of 200 and 400 mg/kg, once daily for 3 days, produced resistance to mechanical pain after 30 minutes. The weight that indicates pain after treatment was significantly increased ( $P < 0.05$  and  $P < 0.001$ ). Aspirin synergies the activity of *M. concanensis* significantly (Table 1).

The ethanolic extract produced a significant reduction in writhing episodes induced by acetic acid (0.6 %) and the percentage protection at 200 and 400 mg/kg was 22.73 % and 51.63 %. The analgesic effect of *M. concanensis* potentates the activity under the same experimental conditions with aspirin as shown by a further reduction in the writhing response and the prevention of abdominal cramping (Table 2). The

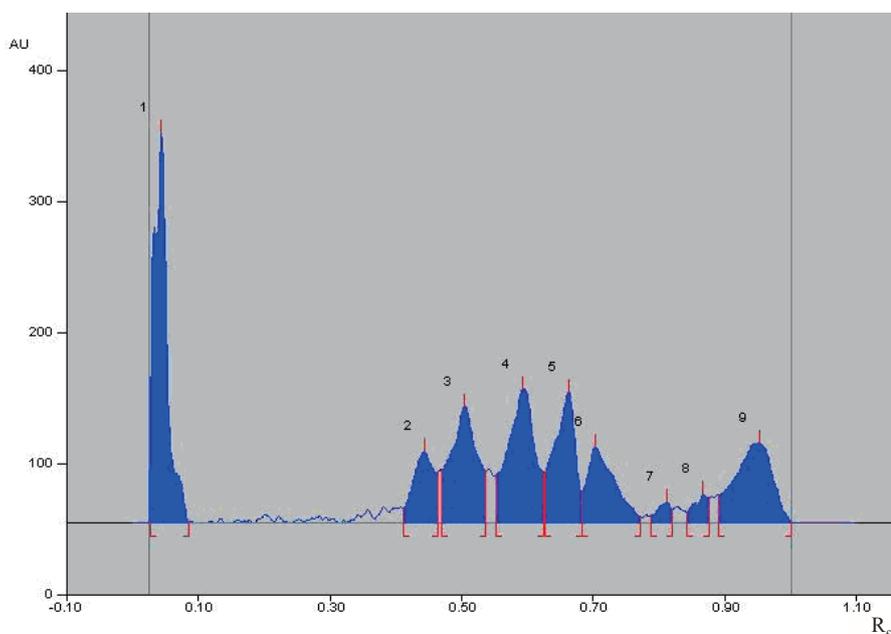


Fig. 1. HPTLC fingerprint profile of *Moringa concanensis* extract scan at wavelength 366 nm.



Table 1. Effect of *M. concanensis* extract on analgesy meter-induced pain in mice \*

Treatment	Dose (mg/kg)	Weight causing pain (g)	
		Before administration	After administration
Control	-	84.62 ± 4.25	85.71 ± 3.82
<i>M. concanensis</i>	200	85.63 ± 3.95	105.02 ± 4.52 <sup>a</sup>
<i>M. concanensis</i>	400	85.90 ± 4.10	129.83 ± 5.33 <sup>b</sup>
Aspirin	25	84.32 ± 3.91	125.52 ± 6.09 <sup>b</sup>
<i>M. concanensis</i> + Aspirin	200 + 25	85.93 ± 3.89	146.64 ± 6.73 <sup>b, z, #</sup>
<i>M. concanensis</i> + Aspirin	400 + 25	83.81 ± 4.13	152.11 ± 7.31 <sup>b, y, #</sup>

\* Values are mean ± S.E.M for six mice

<sup>a</sup>  $P < 0.05$  and <sup>b</sup>  $P < 0.001$  compared to respective control group

<sup>y</sup>  $P < 0.05$  and <sup>z</sup>  $P < 0.001$  compared to respective *M. concanensis* group

<sup>#</sup>  $P < 0.05$  compared to respective aspirin group

Table 2. Effect of *M. concanensis* extract on acetic acid-induced writhing in mice \*

Treatment	Dose (mg/kg)	Number of writhing	% Inhibition
Control	-	22.35 ± 4.61	-
<i>M. concanensis</i>	200	17.27 ± 3.32	22.73
<i>M. concanensis</i>	400	10.81 ± 2.20 <sup>a</sup>	51.63
Aspirin	25	9.56 ± 1.12 <sup>b</sup>	57.23
<i>M. concanensis</i> + Aspirin	200 + 25	6.75 ± 1.40 <sup>b, z</sup>	69.80
<i>M. concanensis</i> + Aspirin	400 + 25	4.07 ± 0.91 <sup>c</sup>	81.79

\* Values are mean ± S.E.M for six mice

<sup>a</sup>  $P < 0.05$ , <sup>b</sup>  $P < 0.01$  and <sup>c</sup>  $P < 0.001$  compared with the respective control group

<sup>z</sup>  $P < 0.01$  compared with the respective *M. concanensis* group

writhing response of the mice to an intra peritoneal injection of anoxious chemical was used to screen for both peripheral and central acting analgesic activity. Acetic acid causes pain by liberating endogenous substances and many others that excite pain at nerve endings [14, 6]. Aspirin and other NSAIDs inhibit cyclooxygenases in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors [15]. It was found that the intensity of the analgesic effect of the ethanolic extract of *M. concanensis* was similar to that of aspirin and probably be due to blockade of the effect or release of endogenous substances that excite pain nerve endings

similar to aspirin and NSAIDs.

Extracts of *M. concanensis* (200 and 400 mg/kg) significantly increased the reaction time and the percentage protection is equivalent to 62.20 % and 125.59 %, respectively. Pentazocine increased the reaction time of *M. concanensis* to 288.77 % and 324.01 % at 200 and 400 mg/kg (Table 3). The hot plate test measures the response to a brief, noxious stimulus and, thus, bears a closer resemblance to clinical pain. The increase in reaction time in the hotplate test confirms the antinociceptive effect of *M. concanensis*. The effect of the extract of *M. concanensis* in terms of analgesic activity may be due to the involvement



Table 3. Effect of *M. concanensis* extract on the hot plate reaction time in mice\*

Treatment	Dose (mg/kg)	Mean latent period (seconds)	
		Initial	After 30 min
Control	-	5.27 ± 1.12	5.08 ± 1.09
<i>M. concanensis</i>	200	5.35 ± 1.41	8.24 ± 1.57 <sup>a</sup>
<i>M. concanensis</i>	400	5.91 ± 1.06	11.46 ± 1.46 <sup>b</sup>
Pentazocine	10	5.58 ± 1.28	16.56 ± 1.08 <sup>c</sup>
<i>M. concanensis</i> + Pentazocine	200 + 10	5.25 ± 1.09	19.75 ± 1.92 <sup>c, z</sup>
<i>M. concanensis</i> + Pentazocine	400 + 10	5.34 ± 1.14	21.54 ± 2.01 <sup>c, z</sup>

\* Values are mean ± S.E.M for six mice

<sup>a</sup>  $P < 0.05$ , <sup>b</sup>  $P < 0.01$  and <sup>c</sup>  $P < 0.001$  compared with the respective control group

<sup>z</sup>  $P < 0.01$  compared with the respective *M. concanensis* group

of endogenous prostaglandins. The fact that *M. concanensis* at the doses tested produced analgesia in all nociceptive models is indicative that it possesses both central and peripheral antinociceptive effects and the mechanism of action of the extracts could, in part, be related to lipooxygenase and/or cyclooxygenase of the arachidonic acid cascade and/or opioid receptors [16, 17].

Treatment with different doses of *M. concanensis* (200 and 400 mg/kg) caused a significant ( $P < 0.05$  and  $P < 0.001$ ) and dose dependent inhibition of the swelling caused by the carrageenan after 3h, equivalent to 26.28 and 44.23 % protection. Under same experimental conditions, the anti-inflammatory effect of nimusilide was 48.08 % at a dose of 50 mg/kg (Table 4). The carrageenan-induced edema is caused by activation of platelet activating factor, prostaglandins and other inflammatory mediators. The carrageenan-induced inflammatory process is believed to be biphasic [18]. The initial phase seen in the first hour is attributed to the release of histamine and serotonin. The second accelerating phase of swelling is due to the release of prostaglandins, bradykinin and lysozymes [19]. Carrageenan-induced edema in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of nonsteroidal anti-

inflammatory agents that primarily inhibit the enzyme cyclooxygenase in prostaglandin synthesis. Based on these reports, it appears that the inhibitory effect of the *M. concanensis* extract on carrageenan-induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

Rheumatoid arthritis is a chronic inflammatory disease affecting about 1 % of the population in developed countries [20]. The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and a pause in body weight gain; during the acute period, the hind and fore paw joint diameters increase [21]. In the later, acute stages of the disease (12 day), rats with adjuvant arthritis are often relatively immobile due to the severity of paw swelling. The use of the adjuvant arthritis model offers an opportunity to study pathological changes in a variety of tissues other than the joints.

Table 5 shows the effect of the ethanolic extract of *M. concanensis* on lysosomal enzymes and glycoprotein in arthritic rats. Lysosomal acid hydrolases play an important role in inflammation associated with rheumatoid arthritis [10]. Glenn *et al.* [22] showed that the addition of nonopsonized *mycobacterium butyricum* to granulocytes obtained from adjuvant inoculated rats causes a significant increase in lysosomal enzyme



Table 4. Effect of *M. concanensis* extract on carrageenan-induced paw edema in rats \*

Treatment	Dose (mg/kg)	Carrageenan-induced hind paw edema volume (ml)	Percentage protection (%)
Control	-	1.56 ± 0.25	-
<i>M. concanensis</i>	200	1.15 ± 0.06 <sup>a</sup>	26.28
<i>M. concanensis</i>	400	0.87 ± 0.04 <sup>b</sup>	44.23
Nimusulide	50	0.81 ± 0.02 <sup>b</sup>	48.08
<i>M. concanensis</i> + Nimusulide	200 + 50	0.73 ± 0.03 <sup>b</sup>	53.21
<i>M. concanensis</i> + Nimusulide	400 + 50	0.71 ± 0.03 <sup>b</sup>	54.49

\* Values are mean ± S.E.M for six mice

<sup>a</sup>  $P < 0.05$  and <sup>b</sup>  $P < 0.001$  compared with the respective control group

Table 5. Effect of *M. concanensis* extract on lysosomal enzymes and glycoproteins in adjuvant-induced arthritis in rats \*

Treatment	Acid phosphatase	N-acetyl glucosaminidase	Total hexose	Sialic acid
<b>Liver homogenate</b>				
Control	0.18 ± 0.02	32.6 ± 2.8	2693 ± 237.4	162.9 ± 15.3
Arthritis	0.32 ± 0.03 <sup>b</sup>	43.9 ± 3.4 <sup>a</sup>	1469 ± 117.7 <sup>c</sup>	103.4 ± 10.1 <sup>b</sup>
Arthritis + <i>M. concanensis</i>	0.19 ± 0.03 <sup>y</sup>	33.5 ± 2.7 <sup>x</sup>	2700 ± 225.6 <sup>z</sup>	160.8 ± 14.6 <sup>y</sup>
<b>Stomach homogenate</b>				
Control	0.13 ± 0.02	26.8 ± 2.4	2532 ± 198.4	149.7 ± 13.6
Arthritis	0.30 ± 0.04 <sup>b</sup>	39.6 ± 3.1 <sup>b</sup>	1695 ± 113.6 <sup>b</sup>	90.2 ± 10.1 <sup>a</sup>
Arthritis + <i>M. concanensis</i>	0.14 ± 0.02 <sup>y</sup>	27.0 ± 2.3 <sup>y</sup>	2559 ± 200.3 <sup>z</sup>	144.0 ± 13.3 <sup>y</sup>

\* Values are mean ± S.E.M for six mice

<sup>a</sup>  $P < 0.05$ , <sup>b</sup>  $P < 0.01$  and <sup>c</sup>  $P < 0.001$  compared with the respective control group

<sup>x</sup>  $P < 0.05$ , <sup>y</sup>  $P < 0.01$  and <sup>z</sup>  $P < 0.001$  compared with the respective arthritis group

release. The increase levels of acid phosphatase and N-acetyl glucosaminidase in the tissue homogenate of the adjuvant-induced arthritic group when compared with the control were significantly reduced following treatment with *M. concanensis* extract in the stomach mucosa ( $P < 0.01$  and  $P < 0.05$ , respectively) and liver ( $P < 0.01$ ) homogenate. These enzymes are involved in the degradation of structural macromolecules in connective tissue and cartilage proteoglycans [23]. Mucus is secreted by the mucus neck cells and covers the gastric mucosa thereby preventing physical

damage and supporting the diffusion of hydrogen ions [24]. The total hexose and sialic acid content in the liver and stomach homogenate was reduced in adjuvant-induced arthritis. The oral administration of *M. concanensis* extract showed a tendency to increase the total hexose ( $P < 0.001$ ) and sialic acid ( $P < 0.01$ ) content suggested a qualitative change in the stomach mucus and liver. Sialic acid was increased and the terms “sialic acid containing glycoprotein” or “sialylated glycoprotein” can be used and mucin can be appropriately termed as sialomucin [25]. The



increased hexose and sialic acid produced by treatment with *M. concanensis* contributes to its defensive effect in treatment of pain and inflammation.

## Conclusion

In conclusion, the results of this study showed that the extract of *Moringa concanensis* possesses analgesic properties and potential anti-inflammatory activity and supports its use in traditional medicine for the treatment of painful inflammatory conditions. This offers a new perspective for the treatment of pain as there is evidence that the symptoms of vital pain vary in intensity depending on the pain threshold.

## Acknowledgement

The authors wish to thank Mr. M. Bhavani Eswaran and Mr. M. K. Singh for technical assistance during the course of the study. These studies were supported in part by grants from Department of Science and Technology, Ministry of S & T, New Delhi. The authors are also grateful to the Director of NBRI for providing necessary facilities.

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