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Modulation of oxidative stress mediators in the liver of adjuvant induced arthritic rats by *Nyctanthes arbor tristis*

Harsha Lad and Deepak Bhatnagar*

Abstract

Background: Oxidative stress is found as one of the causes in various pathological conditions. It has been proven in many studies that medicinal plants have antioxidant constituents that help in coping with the oxidative stress. Therefore, the present study aimed at evaluating the antioxidant effects of *Nyctanthes arbor tristis* leaves extract on the liver of Freund's complete adjuvant (FCA) induced arthritis (RA) rat model.

Methods: The hydroethanolic extract of *N. arbor tristis* leaves (NATE) was evaluated for its phytoconstituents and in vitro antioxidant activity such as free radical scavenging activity (FRSA), metal chelation activity (MCA), reducing power (RP) and plasmid nicking assay. Effects of NATE treatment on the oxidative stress markers along with histopathology of liver in arthritic rats were studied.

Results: The results revealed the presence of phenolic, triterpenoid and flavonoid compounds in hydroethanolic extract of *N. arbor tristis,* which were strongly and positively correlated with the antioxidant activities. NATE significantly modulated oxidative stress markers of liver in favor of reducing the oxidative stress.

Conclusion: It is concluded that increased oxidative stress in liver of FCA induced arthritis mitigated by NATE treatment by strengthening the intracellular antioxidant defense. The study suggests that the leaves of *N. arbor tristis* possess a notable amount of the phytoconstituents which are responsible for its antioxidant effects. The study has provided information about the antioxidant activity of *N. arbor tristis*, which will be useful to develop pharmaceuticals for the treatment of arthritis.

Keywords: *Nyctanthes arbor tristis*, Freund's complete adjuvant, Phytoconstituents, Antioxidant, Plasmid nicking assay

Background

Oxidative stress is the result of imbalance between the generation of reactive oxygen and nitrogen species (ROS and RNS) and the capacity of intracellular antioxidant defense to neutralize them. These are highly reactive and transient species, which include super oxide radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), nitric oxide radical ($NO^{\bullet}$) and many others are generated from the interaction of these species with other radicals or molecules [1]. The excessive generation of these reactive species beyond the capacity to neutralize them can alter the intracellular homeostasis and oxidatively modify various biomolecules such as lipids, proteins and nucleic acids. The oxidative modification results in the alteration of their biological functions leading to various diseases such as cancer, neurodegenerative and inflammatory diseases, including rheumatoid arthritis (RA). These species are formed during various metabolic reactions such as in the electron transport chain during aerobic oxidation, phagocyte activation during inflammation and other reactions. To combat with such oxidative alterations, the cells are equipped with an intracellular antioxidant defense, which includes various enzymes such as super oxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and molecules such as glutathione (GSH) that help in removing such reactive species and maintains intracellular redox status [2]. The oxidation of lipid molecules if not stopped by antioxidants; may initiate a

* Correspondence: deepakbioch@yahoo.co.in
School of Biochemistry, Devi Ahilya University, Khandwa Road, Indore 452001, M.P., India

chain of oxidation of molecules known as lipid peroxidation (LPO). Malondialdehyde (MDA) the end product of LPO, may interact and crosslink with various biomolecules and damage them [3]. Elevated level of MDA with altered activity of oxidative enzyme and antioxidant level was observed in RA sera and synovial fluids [4]. The ROS and inflammatory mediators generated at the site of inflammation may reach to other vital organs such as liver by systemic circulation and affect their normal functioning.

Antioxidant therapy has been shown to ameliorate the oxidative and arthritic changes [5]. The use of herbal plants and their active constituents in the management of various diseases has increased due to its medicinal importance, easy availability, less side effects and low cost. In the Indian medicinal system, a large number of herbal plants and their preparation have been reported for various medicinal usages. It is expected that phytochemicals with potential antioxidant activity may be used for the treatment of diseases in which oxidative stress is one of the causes.

Nyctanthes arbor-tristis is a common ornamental plant of family oleacea and different parts of the plants are used in traditional medicines in India. *N. arbor-tristis* showed the presence of phytosterols, phenolics, tannins, flavonoids, glycosides and saponins with glycosides and alkaloids. Iridoid glycosides in the leaves showed anti-inflammatory and anti-pyretic activities [6]. Various biological activities have been shown in the leaves of *N. arbor-tristis* such as anti-arthritic, hepatoprotective, anti-leishmanial, anti-viral, and anti-fungal activities, as well as analgesic, antipyretic and ulcerogenic activities [7, 8]. In the present study, *N. arbor-tristis* (NATE) was evaluated for its antioxidant effects on the liver of FCA induced arthritic rats. This might be the first report on the effects of *N. arbor-tristis* on the liver antioxidant status in experimental arthritis.

Methods

Chemicals

Ursolic acid, quercitrin hydrate, 1,1,3,3 tetraethoxypropane (TEP), 1-chloro, 2, 4 dinitrobenzene (CDNB), 2, 2-diphenyl-1-p-picryl hydrazyl (DPPH) were purchased from Sigma and Aldrich Co. (St. Louis, MO, USA), while Freund's Complete Adjuvant (FCA) was procured from Difco Laboratories, Detroit, Michigan, USA. Ethanol, Folins Ciocalteau reagent, trichloroacetic acid (TCA) and HPLC grade acetonitrile were purchased from Merck Chemicals, Mumbai, India. Butylated hydroxy toluene (BHT), ferrozine, pyrogallol, ethylenediamine tetracitic acid (EDTA) and other chemicals and solvents of AR grade were purchased from Hi Media Co. Mumbai, India.

Preparation of the extract

The extract of *N. arbor tristis* (NATE) was prepared by adding 1 g powder of dried leaves in 25 ml of ethanol: water (1: 1) at room temperature and kept overnight in the dark. The mixture was centrifuged at 13,000 × g for 45 min. Supernatant obtained was evaporated at room temperature and the yield was calculated. The yield of hydroethanolic extract of *N. arbor tristis* leaves was 24% (w/w). The extractable compound was dissolved in 50% ethanol and used as a test sample for further examination.

Phytoconstituents

The total phenolic, flavonoid and terpenoid content of NATE was determined as described earlier in Lad et al. [9]. Briefly, the total phenolic compounds were estimated using the Folin-ciocalteu reagent and amount of phenolic compounds was calculated using propyl gallate (1 mg/ml in distilled water) as standard. Total triterpenoid content (TTC) was estimated using ursolic acid (1 mg/ml in methanol) as standard. To determine the total flavonoid content (TFC), the aluminium chloride method was followed and quercitrin hydrate (1 mg/ml in ethanol) was used as standard.

In vitro antioxidant activity

Free radical scavenging activity (FRSA) using DPPH, metal chelation activity (MCA) and reducing power of NATE were measured by the method as described earlier [9]. Briefly, The FRSA is based on the reduction of DPPH by the reductants present in the test sample and the decrease in DPPH absorption at 517 nm was measured after 10 min using butylated hydroxyl toluene (BHT) as a reference compound. The chelation of Fe^{2+} by the extract was measured and expressed as % metal chelation activity using EDTA as a reference compound. The reduction of $FeCl_3$ in the presence of test sample was measure of reducing ability.

Plasmid nicking assay

The inhibition of hydroxyl radical induced DNA damage in presence of the extract was measured by the method as described in Lad et al. [9]. Briefly, the native conformation of plasmid DNA (pBR 322) is supercoiled form, which turns into open circular or nicked form in the presence of an oxidizing agent. The inhibition of hydroxyl radical induced DNA damage in presence of the extract is a measure of antioxidant activity. The plasmid DNA (1 μg) was incubated with two different concentrations of NATE (25 and 50 μg) and Fenton's reagent (23 μM of H_2O_2, 376 μM of ascorbic acid and 600 μM of $FeCl_3.6H_2O$). The reaction mixture was incubated for 45 min at 37 °C. BHT (50 μg) was used as a positive control. After incubation, samples were mixed with 5 μl

of loading dye and electrophoresed in 1% agarose gel containing ethidium-bromide (5 μg/ml) by using a 1X TAE buffer, pH 8. Ethidium-bromide stained DNA bands were visualized and recorded in gel documentation system.

Correlation

The correlation coefficient between the phytoconstituents and various antioxidant properties were determined.

Toxicity study and dose selection

Albino Wistar rats of both the sex, weighing in the range of 150–180gm were divided into five groups having three rats in each group. Increasing doses such as 0.0, 1.0, 2.0, 3.0 and 5.0 g/kg body weight of *N. arbor tristis* leaves extract (NATE) were orally administered to different groups of rats and were critically observed for clinical signs, gross behavioral changes and mortality up to 72 h period as per OECD guidelines. Rathod et al., [10] showed dose dependent in vivo antioxidant potential of *Nyctanthes arbor tristis* leaves (50, 100 and 200 mg/kg body wt.). The dose of 200 mg/kg was found to be more effective therefore, was also selected for the present study.

Animals and induction of arthritis

Male albino rats of Wistar strain weighing 180–200 g were used in the study. Rats were housed in polypropylene cages with six animals per cage and maintained under standard laboratory conditions [temperature (25 ± 2) °C and relative humidity 50 ± 4%] with dark and light cycle (12/12 h) and had free access to diet and drinking water. The experiments were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (No. Biochem/01/2013–14). Freund's complete adjuvant (FCA, 10 mg/ml) was used to induce experimental arthritis. FCA (0.1 ml) was subcutaneously injected to the plantar surface of right hind paw of the rats. The rats in which inflammation was consistent up to day 14 of arthritis induction were selected for further studies. Four replicates of six animals in each group (24 rats in each group) were grouped as follows:

Group 1: Control (Ethanol: water (1:1), 2.5 ml/kg body weight [Bw]).
Group 2: FCA injected arthritic rats
Group 3: FCA injected arthritic rats + indomethacin (10 mg/kg Bw/day, p. o. by gavage) from day 15 to day 28 of arthritis induction.
Group 4: FCA injected arthritic rats + NATE (200 mg/kg Bw/day, p. o. by gavage) from day 15 to day 28 of arthritis induction.

Collection of biological samples and preparation for tissue homogenate

The animals were sacrificed by cervical dislocation on day 29 and the liver was removed immediately and washed with ice cold 0.1 M phosphate buffered saline (PBS, 1:9), pH 7.4. A small piece of liver was fixed in 10% formalin for histopathology. The tissue was blotted dry, weighed and minced to prepare tissue homogenate. A 10% tissue homogenate was prepared in 0.1 M PBS using Potter-Elvehjem Homogenizer (Remi, Mumbai, India) followed by centrifugation at 16,000 × g for 25 min at 4 °C. The supernatant obtained was immediately used for the antioxidant enzyme assay, GSH and carbonyl content.

Histopathology

Histopathology was performed to study the effect on the cellular architecture of the liver in arthritic and treated rats. The haemotoxylin and eosin (H and E) stained sections were visualized under a light microscope (Nikon eclipse 50i, Japan) under 20X.

Assay for lipid peroxidation

Lipid peroxidation (LPO) in liver was determined by measuring malondialdehyde (MDA) content using HPLC (Younglin, Korea). The conditions for HPLC were similar as described by Tukozkan et al. [11]. The MDA peak was identified and quantified in the samples using standard calibration curve obtained from 1,1,3,3 tetraethoxypropane (TEP), using the linear regression equation derived from the peak area and expressed as nmol MDA/mg protein.

Assay of antioxidant enzymes

The antioxidant enzyme activity, GSH and carbonyl content were measured according to the method as described earlier in Lad et al. [12]. The ability of the enzyme to inhibit the autoxidation of pyrogallol in the presence of EDTA was used as a measure of SOD activity at 340 nm. The decomposition of hydrogen peroxide was used to measure catalase activity. The method for the estimation of glutathione-S-transferase (GST) activity was based on the property of conjugation of –SH group with 1-chloro 2, 4-dinitrobenzene (CDNB) mediated by GST. Estimation of tissue glutathione (GSH) was based on the development of a relatively stable yellow color with 5, 5'-dithio-bis-2, nitrobenzoic acid (DTNB) with GSH. Protein carbonyl content in liver was determined after derivatisation with 2, 4 - dinitrophenyl hydrazine (DNPH) and measured at 370 nm using molar absorption coefficient of 22,000 M^{-1} cm^{-1}.

Statistical analysis

The data obtained was analyzed by one-way analysis of variance (ANOVA) followed by the Tukey's (HSD) test using SPSS version 20.0 to calculate interrelation between the groups. The correlation was calculated using Pearson's correlation coefficient.

Results

Phytochemical screening

The total phenolic, triterpenoid and flavonoid contents of NATE were determined. The phenolic, triterpenoid and flavonoid contents were 29.74 ± 0.13 mg of gallic acid equivalent (GAE)/g of extractable compound, 184.43 ± 1.13 mg ursolic acid equivalent (UAE)/g of extractable compound and 162.13 ± 1.52 mg quercitrin hydrate equivalent (QHE)/g of extractable compound respectively (Table 1).

In vitro antioxidant activity

NATE was screened for its in vitro antioxidant activity using various in vitro assays. The extract showed a concentration dependent increase in FRSA, MCA and RP (Fig. 1a-c).

Plasmid nicking assay

The Fenton's reagent mediated plasmid DNA damage was assessed with and without extract (Fig. 2). The native confirmation of plasmid DNA pBR322 was supercoiled form (Fig. 2, Lane 1). The hydroxyl radicals generated in Fenton's reaction caused DNA damage as evidenced by transforming its native supercoiled form to open circular or linear form (Lane 2). The result of the plasmid nicking assay showed that in the presence of BHT (Lane 3, 50 μg) and NATE (Lane 4 and 5; 25 and 50 μg respectively), the native supercoiled form was retained. The protection provided by NATE was concentration dependent and was comparable with BHT.

Correlation study

The results showed a strong and positive correlation between the phytoconstituents and the antioxidant properties (Table 2).

Table 1 Estimation of total phenolic, triterpenoid and flavonoid content of NATE

Constituents	Content
Total phenolic conntent[a]	29.74 ± 0.13
Total triterpanoid content[b]	184.43 ± 1.13
Total flavonoid content[c]	162.13 ± 1.52

Values are mean ± SE of four replicates
[a] = mg of gallic acid equivalent/g extractable compound
[b] = mg of ursolic acid equivalent/g extractable compound
[c] = mg of quercitrin hydrate equivalent/g extractable compound

Toxicity study

The result of toxoicity study showed no toxic or deleterious effects upto 5.0 g/kg oral dose. As the rats were administered the maximal possible dose, the LD50 value of NATE could not be determined.

Determination of LPO

The results showed that MDA content was significantly higher in FCA induced arthritic rats (Table 3; Group 2) as compared to control (Group 1). A significant reduction in MDA level was observed after indomethacin (Group 3) and NATE (Group 4) administration when evaluated against arthritic control (Group 2) and was comparable to control (Group 1).

Determination of antioxidant enzyme activities

The decrease in liver SOD, CAT and GST activities was significant in arthritic animals (Table 3; Group 2) as compared to control rats (Group 1). The SOD and GST activity was found to increase after indomethacin and NATE treatment (Group 3 and 4) with respect to arthritic group (Group 2) and was comparable with control (Group 1). The increase in liver CAT activity was not significant in indomethacin and NATE treated rats (Group 3 and 4) as compared to control (Group 1) and arthritic group (Group 2).

The results showed that liver GSH content was not significantly decreased in arthritic rats (Table 3; Group 2) as compared to control (Group 1). Treatment with indomethacin (Group 3) and NATE (Group 4) significantly increased the GSH content as compared to arthritic rats (Group 2) and was comparable with the control (Group 1).

The liver carbonyl content was significantly elevated in arthritic group (Table 3; Group 2) as compared to control (Group 1). The carbonyl content was significantly decreased by indomethacin and NATE treatment (Group 3 and 4) as compared to arthritic rats (Group 2).

Effect on histopathology of liver in arthritic and treated groups

The animals from control group (Fig. 3a) showed the normal cellular structure of liver at centrilobular zone. In contrast to this, the liver from the arthritic group (Fig. 3b) showed anomalous changes in cellular morphology when compared to control. The histology of liver from FCA induced animals revealed moderate microvesicular fatty changes, with sinusoidal congestion and infiltration of mononuclear cells. Indomethacin treatment also showed sinusoidal congestion with microvesicular fatty changes as compared to control (Fig. 3c). NATE treatment (Group 4) protected normal cellular characteristics of liver by reducing fatty changes and

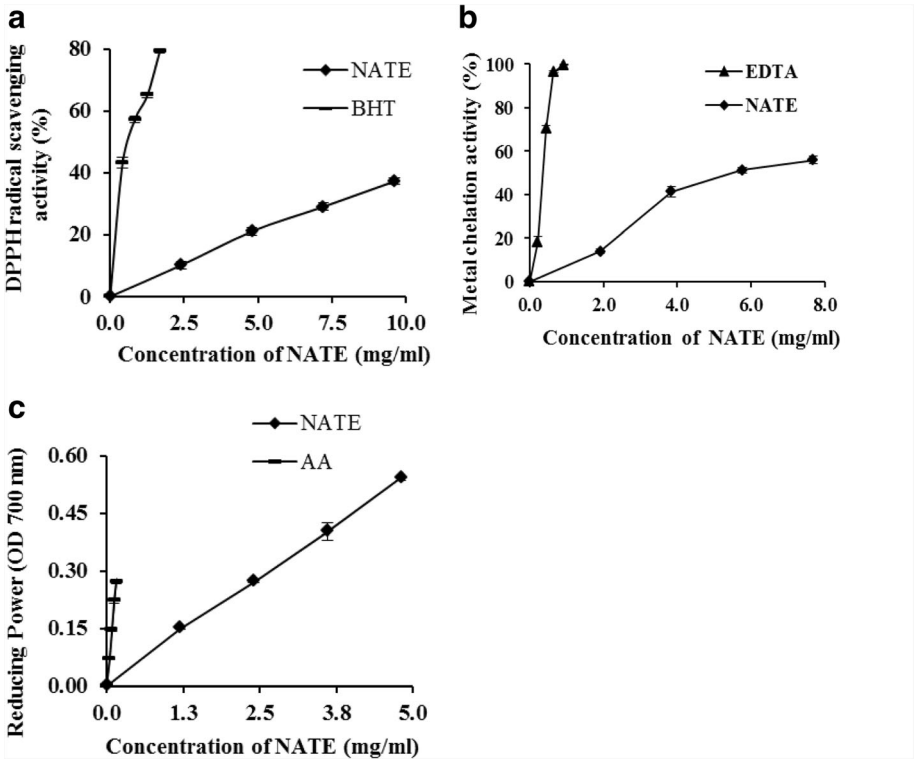

Fig. 1 Effects of different concentrations of NATE on (**a**) DPPH radical scavenging activity; (**b**) Metal chelation activity; (**c**) Reducing power. Values are Mean ± SE of 4 replicates. All experimental values were significant at $P < 0.05$ as compared to controls

sinusoidal congestion with mild mononuclear cell infiltration (Fig. 3d).

Discussion

Free radical mediated oxidative stress due to the compromised intracellular antioxidant defense has been one of the causing factors behind the etiology of a wide range of clinical disorders. Therefore, limiting the free radical generation or scavenging them may serve as a useful means in the therapy of various diseases. Antioxidant therapy may be useful in this regard and have shown promising results [5]. The use of antioxidants from natural sources will help to combat the diseases and to develop new therapeutic candidate.

In the present study, phytochemical analysis of NATE revealed the presence of phenolic, triterpenoid and flavonoid content. In an earlier study, the total phenolic and flavonoid content of *N. arbor trisitis* leaves 98.56 ± 0.46 mg/g and 34.51 ± 0.45 mg/g respectively was reported [13]. The difference in the amount of phytoconstituents in the present study was due to the difference in solvent and the extraction procedure. These compounds are potent antioxidants that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the initiation step. The TPC and TFC exhibit redox properties due to the presence of polyphenolic groups that allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers and can be used as important

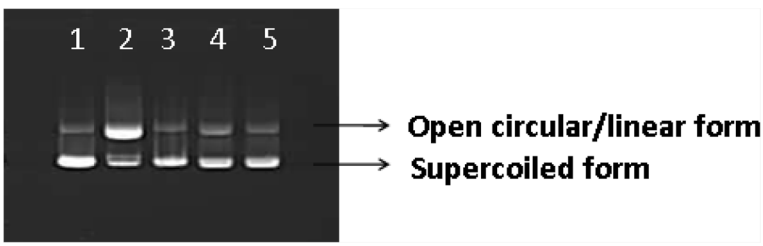

Fig. 2 Plasmid nicking assay Lane 1: DNA; Lane 2: DNA + Fenton's reagent; Lane 3: DNA + BHT (50 μg) + Fenton's reagent; Lane 4: DNA + NATE (25 μg) + Fenton's reagent; Lane 5: DNA + NATE (50 μg) + Fenton's reagent

Table 2 Correlation coefficient between phytoconstituents and antioxidant properties of NATE

Antioxidant properties Phyto-Constituents	FRSA	MCA	RP
TPC	1.00*	0.967*	0.995*
TFC	0.998*	0.967*	0.995*
TTC	0.998*	0.967*	0.995*

FRSA Free radical scavenging activity
MCA Metal chelation activity
RP Reducing power
*Correlation coefficient is significant at $P < 0.05$

indicators of the antioxidant capacity [14]. These polyphenols have been found to associate with several health benefits [15].

The DPPH has been used to evaluate the antioxidant activity of the test sample. It is evident that the hydrogen donating ability of a substance is responsible for its free radical scavenging activity (FRSA). The result of the DPPH assay suggested that NATE possessed FRSA, which may contribute to its in vivo antioxidant potential as has also been reported by Balasundari et al. [16]. The polyphenolic compounds exhibit antioxidant activity by forming a metal ion complex, thus inhibiting the free metal ion induced radical generation [17]. The reducing power measures the ability of the antioxidant to donate electron to reduce Fe^{3+} to Fe^{2+}. The reports suggested that the plant extract with greater TPC was associated with their reducing power [18]. The reducing power of NATE is suggested to be due to the presence of flavonoids and phenolic acids, capable of reducing Fe^{3+} to Fe^{2+}. The reducing ability of the extract is found to associate with the presence of reductones that is supposed to be responsible for antioxidant activity by donating a hydrogen atom thus interrupting the free radical chain reaction [19]. An earlier report by Savarimuthu et al [20] has shown the presence of various in vitro antioxidant activities in the leaf extract of *N. arbor trisitis* which is in agreement with the present study.

In a radical generating system the change in conformation of plasmid DNA from supercoiled to open circular or linear form is indicative of oxidative DNA damage, which was inhibited in the presence of a radical scavenger [21]. In the present study, the native conformation i.e. supercoiled form of DNA was retained in the presence of NATE in a concentration dependent manner, which was comparable to the protection provided by BHT. It indicated that NATE has ability to chelate free metal ions and to scavenge the free radicals and thus protected the DNA from oxidative damage, which can be attributed to its phytoconstituents. These antioxidant properties can be correlated with phytoconstituents such as total phenolic, flavonoid and triterpenoid content of the extract. The correlation study showed a strong and positive correlation between phytoconstituents and antioxidant properties. Various other studies have indicated a linear relationship between the major phytoconstituents such as total phenolic compounds and antioxidant activity [9, 22, 23]. A study on Chinese medicinal plants has also shown a positive correlation between antioxidant activity and total phenolic content [24].

Determination of acute toxicity is necessary to evaluate the toxic effects after oral administration of a single dose of the drug. NATE did not show any toxic or deleterious effects upto the dose of 5.0 g/kg indicating low toxicity of the extract at high doses. As the rats were administered upto maximal possible dose, the LD50 value of NATE could not be determined, which is in agreement with the study reported by Bhalerao et al. [25].

In pathological conditions the balance between the free radical generation and the antioxidant activity is lost, resulting in oxidative damage to the cells and tissues. Though rheumatoid arthritis (RA) is an inflammatory disease of joints, its systemic effects have also been observed [26, 27]. Many reports showed the involvement of the liver in RA. The liver from adjuvant induced arthritis showed elevated oxygen uptake, decreased gluconeogenesis, increased glycolysis and reduced metabolism of xenobiotics [28, 29]. It is known that activated

Table 3 Effect of NATE treatment on LPO, antioxidant enzymes, GSH and carbonyl content in the liver of FCA induced arthritic rats

Group	Treatments	LPO[a]	SOD[b]	CAT[c]	GST[d]	GSH[e]	Carbonyl[f]
1	Control	0.12 ± 0.05	15.05 ± 1.18	393.65 ± 20.37	0.86 ± 0.09	41.01 ± 3.98	1.78 ± 0.42
2	RA	0.43 ± 0.04*	8.31 ± 0.37*	286.42 ± 20.25*	0.57 ± 0.05*	32.01 ± 2.05^{NS}	4.40 ± 0.22*
3	Indomet	0.09 ± 0.05$^{\#}$	12.43 ± 0.61$^{\#}$	379.41 ± 41.43^{NS}	0.94 ± 0.07$^{\#}$	44.76 ± 0.92$^{\#}$	2.25 ± 0.09$^{\#}$
4	NATE	0.18 ± 0.04$^{\#}$	11.95 ± 1.07$^{\#}$	337.96 ± 27.60^{NS}	0.98 ± 0.10$^{\#}$	41.13 ± 1.82$^{\#}$	1.70 ± 0.19$^{\#}$

Values are Mean ± SE of four replicates with six animals in each group
Groups 2, 3 and 4 as compared to Group 1. *$P < 0.05$
Groups 3 and 4 as compared to Group 2. $^{\#}P < 0.05$; ^{NS} Not significant
[a] = nmoles MDA formed/mg protein
[b] = units/mg protein
[c] = μmoles H_2O_2 decomposed/min/mg protein
[d] = μmoles GSH conjugated/min/mg protein
[e] = μmoles DTNB conjugated/mg protein
[f] = nmoles of carbonyl formed/mg protein

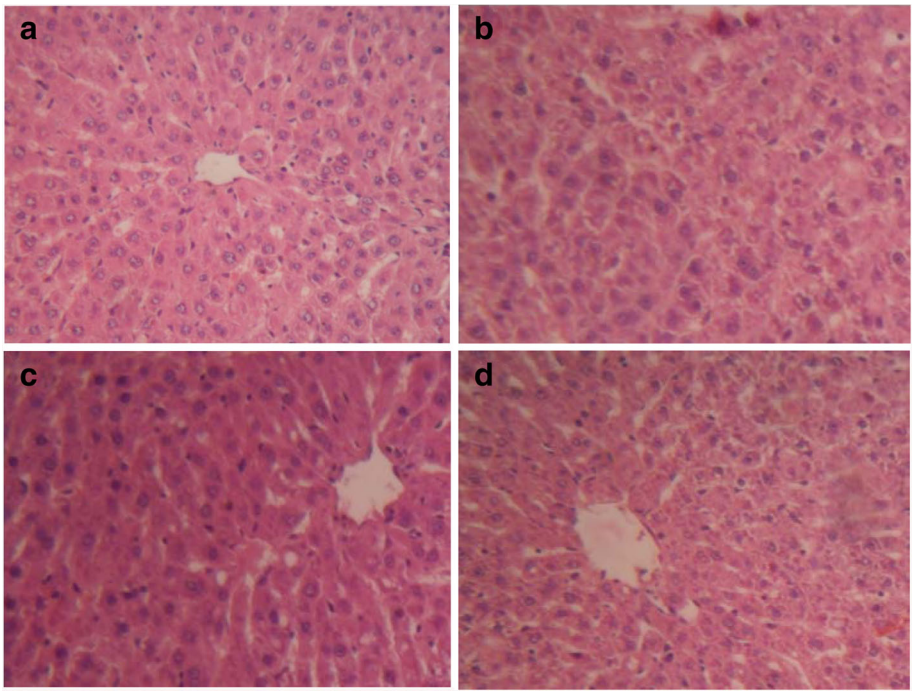

Fig. 3 H and E stained section of liver from (**a**) control rats; (**b**) FCA induced arthritic rats; (**c**) FCA induced arthritic rats treated with Indomethacin (10 mg/kg) and (**d**) FCA induced arthritic rats treated with NATE (200 mg/kg)

inflammatory cells lead to ROS production in rheumatoid arthritis [30]. The tissue damage resulting from the inflammatory effects of RA might be due to increased LPO and prostaglandin synthesis [31]. The elevation of the MDA content may be the result of LPO, which is a free radical driven reaction that causes tissue membrane damage by reaction of oxygen with polyunsaturated fatty acids (PUFAs) [32]. Hsouna et al. [33] associated the reduced activity of antioxidant enzymes with the enhancement in the lipid peroxidation. In the present study, elevated levels of MDA in liver of arthritic animals have been observed, which might be due to tissue membrane damage. The treatment with NATE was found to inhibit LPO in arthritic animals, which gives evidence that NATE works as a strong antioxidant and plays an important role in improving free radical mediated tissue damage as indicated by reduced levels of MDA in liver of NATE treated group.

The inhibition of liver SOD may also be due to free radicals produced during the oxidative stress. However, if free radicals are not sufficiently scavenged by antioxidants, it may reduce the total antioxidant capacity in arthritic animals and lead to cellular injury. The increased activities of SOD in NATE treated animals may be a regulatory response to decreased oxidative stress. It has been shown that low SOD levels in RA patients, increased after NSAID treatment and reached normal levels [34]. The superoxide anions are dismutated by SOD to form H_2O_2, which is decomposed by CAT [35]. The decrease in liver catalase activity may be due to its inactivation by H_2O_2. GSH in the liver has a redox system, which plays the first line of defense against various exogenous and endogenous compounds. GSH has an important role both as a substrate for glutathione peroxidase (GPx), glutathione reductase (GR) and cofactor for GST [36, 37]. There was a significant increase in GSH content of liver in arthritic rats after NATE treatment which was comparable to control. This may be due to the steady supply of GSH from the liver to other organs [38]. However, the activity of GST was found to significantly increase in NATE treated group, probably due to up regulation of GST by the phytoconstituents of NATE. The improved activity of antioxidant enzymes after NATE administration may be due to the free radical scavenging and reducing ability of the extract. Protein carbonyls are the most commonly used markers of protein oxidation and have been reported in many human pathologies [39, 40]. It is suggested that the free radical scavenging and reducing power of NATE may minimize the oxidative effects on proteins.

The mild inflammatory and fatty changes in the liver of arthritic rats were in agreement with the study by Fernandes et al. [41]. The result suggests that NATE treatment showed recovery in the liver by controlling the infiltration of mononuclear cells and fatty changes.

Conclusion

The results of the study suggest higher oxygen free radical production in the liver of arthritic animals as evidenced by increased MDA and carbonyl content with modulated antioxidant enzyme activity in RA. The present study revealed that the leaves of *N. arbor tristis* possessed potential antioxidant properties. It is suggested that treatment with *N. arbor tristis* strengthen the intracellular antioxidant defense by its antioxidant phytoconstituents to cope up with the free radical mediated oxidative stress in the liver of arthritic rats.

Authors' contributions

HL: designed and performed the experiments, participated in data analysis and manuscript preparation. DB: participated in study design and helped in manuscript preparation. Both authors read and approved the final manuscript.

Competing interests

Harsha Lad and Deepak Bhatnagar declare that they have no competing interests.

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