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Evaluation of anti-arthritic activity of syringic acid: A natural compound isolated from plants

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Abstract
Aim: Effect of Syringic Acid on Lysosomal and Anti-oxidant Enzymes in Arthritic Animals**.**
Material and Methods: The acute oral toxicity studies were carried out according to the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guideline 423. The Wistar albino rats were divided into 5 groups of six animals in each. For the induction of chronic inflammatory response, FCA (0.1 ml) was injected through intra-articular injection in left ankle joint of rats on 0 day. Pre-induction baseline was taken prior to the injection of Freund's Complete Adjuvant (FCA) measured by left paw volume of each animal at 0 day for the induction of arthritis in Wistar rats. The treatments with Syringic acid were given once daily from day of injection to 21st day. The severity of adjuvant arthritis was quantified by measuring the volume of the hind paw using Plethysmograph. Body weight was measured of all groups at zero days before immunization and at 21st day after treatments over by using a single pan weighing balance.
Results: No toxic effects were observed at a higher dose of 500 mg/kg body weight of Wistar rats. Hence, 1/ 10th dose was selected as effective dose or therapeutic dose. The cut off value of 50 and 1/5 dose double of 100 mg/kg were selected for anti-arthritic and anti-inflammatory activity. The assessment made on the 21st day showed that the Syringic acid treatments at both doses (low and high) had moderately significant and highly significant effect and reduced ($p< 0.01$& $p<0.001$) the adjuvant-induced lesions in the respective treatment groups as compared with the arthritis control group. Syringic acid had moderately and highly significant increase in body weight ($p<0.01$ & $p<0.001$) as compared to arthritic rats. Administration of Syringic acid at dose of 100 mg/kg caused highly significant decrease ($p<0.001$) in MDA levels and increase in GSH and SOD activities.
Conclusion: In conclusion, it can be stated that the Syringic acid have beneficial effects in long lasting reduction in rat paw edema, arthritic index and various lysosomal enzymes. It also showed a protective effect on arthritic rat joints without any toxic effect.

Keywords: syringic acid, lysosomal and anti-oxidant enzymes, SOD, LDH, glutathione, rheumatoid arthritis

Introduction
RA is one of many autoimmune diseases that predominate in females. The ratio of female to male patients may vary from 2:1 to 4:1. Pregnancy usually is associated with remission of the disease with subsequent relapses after delivery (Nagy, 2007). The annual incidence of RA is estimated to be about 30 per 100,000 and it affects approximately 0.5%–1.0% of the world population (Alamanos *et al.*, 2006) [1].
Clinically, RA is characterized by Polyarthritic, swelling and, in many cases, manifests extra- articular involvement. In the early stage of the disease, typical signs and symptoms are swelling and pain of the proximal interphalangeal and metacarpophalangeal joints. Later, the larger joints become affected, especially those of the arms, feet and knees. In addition, RA can affect other systems of the body, and this may range from rheumatoid nodules to life- threatening vasculitis (Smolen & Steiner 2003) [22].
The investigation of natural products as source of novel therapeutic agents has reached its peak in the western pharmaceutical industry in the period 1970-1980, which resulted in the synthesis of non-synthetic compounds or molecules. Of the 877 small-molecule New Chemical Entities (NCEs) introduced between 1981-2002, roughly half almost 49% were natural products, semi-synthetic natural product analogues or synthetic compounds based on natural products (Koehn & Carter, 2006).
As per the literature review, it has been observed that syringic acid is listed among the various medicinal plants widely been used in the acute and chronic inflammatory conditions. In the absence of any scientific evidence for their anti-arthritic activity in chronic inflammatory conditions and effect on lysosomal enzymes, there is a need in scientifically establishing the anti-arthritic activity so that we are able to come up with a more effective and potent bioactive phytoconstituents with less side effects in comparison with existing synthetic drugs.

Materials and Methods
Phytoconstituents (Syringic Acid)
The Syringic Acid was purchased from the local market.

Preparation of Solution
For the animal studies, test solution of Syringic acid was prepared in Tween 80 in a concentration of 1%.

Preliminary *in-vivo* anti-arthritic activity
Selection of animals
Wistar albino rats of either sex between 2 and 3 months of age weighing 150-200 g were used which were procured

from the central animal house of India. All animals were housed in an animal room under normal condition of 25±1^{0}C, 12-h light and dark cycle. All the experimental procedures were carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The study designs were approved by the Institutional Animal Ethical.

Acute toxicity studies

The acute oral toxicity studies were carried out according to the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guideline 423. Syringic acid solution was prepared as a suspension by triturating dried compound with 1% tween 80, prepared in distilled water. Wistar rats (150-200 g) were used for acute toxicity study to determine the acute toxicity. One tenth and one fifth of the lethal dose was taken as effective dose (therapeutic dose) and cut off value was selected as 50 and 100 mg/kg to evaluate the dose dependent action for the evaluation of anti-arthritic activity (OECD guidelines, 2001) [18].

Evaluation of anti-arthritic activity

The Wistar albino rats were divided into 5 groups of six animals in each. For the induction of chronic inflammatory response, FCA (0.1 ml) was injected through intra-articular injection in left ankle joint of rats on 0 day. Pre-induction baseline was taken prior to the injection of Freund's Complete Adjuvant (FCA) measured by left paw volume of each animal at 0 day for the induction of arthritis in Wistar rats. The treatments with Syringic acid were given once daily from day of injection to 21st day. The animal groups are as follows (Arulmozhi *et al*., 2011) [2].

Group-I: Normal control, treated with Saline solution on zero day

Group-II: Arthritic control, treated with 0.1 mL of FCA on zero day.

Group-III: Standard control: treated with prednisolone (10 mg/kg, p.o.) + FCA

Group-IV: Treated with Syringic Acid (50 mg/kg, p.o.) + FCA

Group-V: Treated with Syringic Acid (100 mg/kg, p.o.) + FCA

Measurements of paw volume

The severity of adjuvant arthritis was quantified by measuring the volume of the hind paw using Plethysmograph. Paw volume (ml) was measured at 0 days and thereafter 4, 8, 12, 16 and 21 days of FCA post-inoculation. Data were expressed as the increase in paw volume with respect to day 0 paw volume. The percentage inhibition of paw volume was measured by following formula (Arulmozhi *et al*., 2011; Ignacimuthu *et al*., 2011) [2, 9].

$$\text{Percentage inhibition} = \frac{Vc - Vt}{Vt} \times 100$$

Where,

Vc-Paw volume of control animals Vt-Paw volume of treated animals

Measurements of body weight

Body weight was measured of all groups at zero days before immunization and at 21st day after treatments over by using a single pan weighing balance (Jalalpure *et al*., 2011) [10].

Measurements of hematological parameters

The number of leukocytes from each rat was determined using a counting chamber (celldyn-1200, Abbott Carepam). Erythrocyte sedimentation rate (ESR) was determined using the Wintrobe method. RBCs and Haemoglobin were determined by routine laboratory method (Jalalpure *et al*., 2011) [10].

Arthritis assessment

The severity of the arthritis in each paw was quantified daily by a clinical score measurement from 0 to 4 as follows: 0 – no macroscopic signs of arthritis (swelling or erythema), 1 – swelling of one group of joints (namely, wrist or ankle joints), 2 – swelling of two groups of swollen joints, 3-swelling of three groups of swollen joints, 4 welling of the entire paw (Arulmozhi *et al*., 2011) [2].

Estimation of various biochemical parameters

At the end of the experimental period, rats were fasted overnight and the anaesthetized rats were sacrificed by cervical decapitation. Cartilages and Liver tissue were removed for estimation. Liver homogenates were centrifuged at 600g for 10 min. The sediment which containing nuclei, unbroken cells and plasma membranes (nuclear fraction) were separated and the supernatant was subjected to centrifugation at 16,000g for 30 min. The sediment was suspended in 0.25M sucrose buffer. Aliquots were withdrawn at 0 and 30 min intervals, immediately cooled at 0 ^{0}C and centrifuged at 16,000g for 30 min. Enzyme activity in the supernatant was determined.

Estimation of alkaline phosphatase (ALP)

0.5 ml of 0.4% p-nitrophenol phosphate substrate and 0.5 ml of glycine buffer (pH 10.5) were added in tubes marked as blank and test. The tubes were placed in water bath at 37^{0}C for 5 min. The reaction was initiated by the addition of 0.1 ml of liver homogenate and distilled water to the test and blank tubes, respectively and the time was noted. Exactly after 30 min incubation at 37^{0}C, the reaction was arrested by the addition of sodium hydroxide. The tubes were mixed well and the colour developed was read at 410 nm against the reagent blank. Then, 0.1 ml of concentrated hydrochloric acid was added to blank and test, mixed well and read at 400 nm against reagent blank. The ALP activity is expressed in terms of micromoles of p-nitrophenol formed per hour per milligram of protein (Walter & Schutt, 1974; Kandaswamy *et al*., 2007) [26, 12, 17].

Estimation of acid phosphatase (ACP)

Into clear glass tubes marked blank and test, 0.5 ml of 0.4% p-nitrophenyl phosphate substrate, 0.5 ml of 1.0 M citrate buffer (pH 4.85) and 0.2 ml of 0.2M DL-tartarate were added. The tubes were incubated at 37^{0}C in a water bath for 5 min. The reaction was initiated by the addition of 0.1 ml of serum or liver homogenate and water to the test and blank tubes, respectively and the time was noted. Exactly after 30 min of incubation at 37^{0}C, the reaction was arrested by the addition of 3.8 ml of 0.1 N NaOH. The reaction product, p-nitrophenol was measured spectrophotometrically at 415 nm against a reagent blank. The enzyme activity was expressed as micromoles of p-nitrophenol formed per hour per milligram of protein (Walter & Schutt, 1974; Kandaswamy *et al*., 2007) [26, 12, 17].

Estimation of lactate dehydrogenase (LDH)
The incubation mixture contained 1.0 ml of buffered substrate and 0.2 ml of enzyme. 0.2 ml of NAD was added to test, mixed and incubated at 37^0C for 15 min. exactly after 15 min, 1.0 ml of DNPH was added to each (test and control) tubes and 0.2 ml of NAD was added to control. Added 5.0 ml of 0.4 N NaOH the colour developed was read immediately at 420 nm. A standard curve with sodium pyruvate solution was also obtained for calibration. The enzyme activity was expressed as micromoles of pyruvate liberated per milligram of protein per minute (Keffer, 1991; Kandaswamy *et al*., 2007) [13, 12, 17].

Estimation of malonaldehyde (MDA)
The thiobarbituric acid-reactive substance was measured as a marker of lipid peroxidation in the articular cartilage. The homogenized tissue was added to 1.5 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetate buffer (pH 3.5) and 1.5 ml of 0.8% TBA (thiobarbituric acid) solution. The mixture was heated at 95 ◦C for 1 h. After cooling, 5ml of n-butanol pyridine (14:1) was added for extraction and the absorbance of n-butanol–pyridine layer at 532 nm (Shimadzu UV Vis 1700) was measured for determination of TBA reactive substance (Kumar *et al*., 2009; Arulmozhi *et al*., 2011) [15, 2].

Estimation of glutathione (GSH)
An aliquot of articular tissue homogenate supernatant (0.4 ml) was added to dark polyethylene tube containing 1.6 ml of 0.4M Tris–EDTA buffer, pH 8.9. After vortex-mixing, 40µl of 10mM dithiobisnitrobenzoic acid in methanol was added. The samples were vortex- mixed again and the absorbance was read at 412 nm after 5 min (Shimadzu UV-Vis 1700). The values of unknown samples were drawn from a standard curve plotted by assaying different known concentrations of glutathione (GSH). The amount of GSH was expressed as µmol/g of protein (Kumar *et al*., 2009; Arulmozhi *et al*., 2011) [15, 2].

Estimation of superoxide dismutase (SOD)
Total SOD activity was measured by determining the ability to inhibit the auto-oxidation of pyrogallol. The rate of auto-oxidation was determined by measuring increases in the absorbance at 420 nm. Reaction mixture containing 0.2mM pyrogallol in 50mM Tris– cacodylic acid buffer (pH 8.5) and 1mM diethylene triamine pentaacetic acid was incubated for 90s at 25^0C. One unit of SOD activity is defined as the amount of the enzyme required to inhibit the rate of pyrogallol auto oxidation by 50% (Kumar *et al*., 2009; Arulmozhi *et al*., 2011) [15,2].

Statistical Analysis
The values are expressed in mean ± SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance. $p< 0.05$ was chosen as the level of significance.

Results
Acute toxicity studies
No toxic effects were observed at a higher dose of 500 mg/kg body weight of Wistar rats. Hence, 1/ 10th dose was selected as effective dose or therapeutic dose. The cut off value of 50 and 1/5 dose double of 100 mg/kg were selected for anti-arthritic and anti-inflammatory activity.

Table 1: Acute toxicity studies of Syringic acid

S. No.	Treatment	Dose (mg/kg)	Number of animals	Mortality			Toxicity Profile
				After 24 hrs	After 7 days	After 14 days	
1	Syringic Acid	500	5	0	0	0	Safe

Freund's complete adjuvant induced rat paw edema
Observations of paw volume were recorded on 4th, 8th, 12th, 16th, 21st day after adjuvant injection. The CFA-induced arthritic control group showed signs of arthritis development, as seen by the increase in the paw volume and other indications, such as decreased body weight, also showed induction of arthritis in the CFA-treated control group rats. The assessment made on the 21st day showed that the Syringic acid treatments at both doses (low and high) had moderately significant and highly significant effect and reduced ($p< 0.01$& $p<0.001$) the adjuvant-induced lesions in the respective treatment groups as compared with the arthritis control group.

Table 2: Effects Syringic acid on paw volume in FCA induced arthritis in rat

S. No.	Groups & Treatments	Paw Volume in mL					
		Zero Day	4th Day	8th Day	12th Day	16th Day	21st Day
1	Normal Control	0.29±0.08	0.31±0.03	0.31±0.04	0.31±0.01	0.31±0.06	0.31±0.14
2	Arthritic Control, 1% Tween 80, p.o.	0.30±0.02	0.50±0.04**	0.85±0.01***	0.92±0.02***	1.31±0.06***	1.63±0.02***
3	Prednisolone 10 mg/kg, p.o.	0.31±0.07	0.33±0.06*	0.40±0.18**	0.45±0.03***	0.52±0.04***	0.55±0.08***
4	Syringic acid, 50 mg/kg, p.o.	0.31±0.10	0.42±0.02	0.50±0.01*	0.60±0.04*	0.68±0.01**	0.74±0.02***
5	Syringic acid, 100 mg/kg, p.o.	0.32±0.01	0.40±0.04	0.47±0.05*	0.55±0.08**	0.60±0.02***	0.65±0.01***

Values are expressed as mean±SEM, n=6 in each group; * p <0.05, compared to disease control ** p<0.01, compared to disease control. *** p <0.001, compared to disease control

Effects on body weight
Although the weights were almost identical in all group of animals at 0 to 7 days during the subsequent course of disease, the body weight always declined in arthritic control group from 14th day to 21st day. In arthritic group, decrease in body weight were observed on the subsequent days, whereas groups treated with standard, Syringic acid showed improvements in body weight.
This effect was observed from 14th day to last day of the experiment as compared to arthritic rats. Syringic acid had moderately and highly significant increase in body weight ($p<0.01$ & $p<0.001$) as compared to arthritic rats.

Table 3: Effects of Syringic acid on body weight in FCA induced arthritis in rat

S. No.	Groups & Treatments	Days	
		Zero	21st
1	Normal Control	190.20±0.78	191.47±0.20
2	Arthritic Control, 1% Tween 80, p.o.	191.40±0.18	165.18±0.20***
3	Prednisolone, 10 mg/kg, p.o.	191.80±0.20	216.30±0.16***
4	Syringic acid, 50 mg/kg, p.o.	191.18±0.40	209.20±0.16**
5	Syringic acid, 100 mg/kg, p.o.	190.20±0.50	213.40±0.08***

Values are expressed as mean±SEM, *n*=6 in each group; *p<0.05, compared to disease control ** p<0.01, compared to disease control. ***p<0.001, compared to disease control

Effects on haematological parameters

FCA-induced arthritic rats at 21st day showed elevation in the total WBC count and reduction in RBC. However, significantly *(p<0.001)* increased ESR while the haemoglobin was significantly *(p<0.001)* reduced in the control group when compared with normal group. However, standard, and Syringic acid had highly significant effects (p<0.001) in recovery of RBCs and haemoglobin. They also showed highly significant effects on decrease in WBCs and ESR.

Table 4: Effects of Syringic acid on haematological parameters in arthritis in rat

S. No.	Groups & Treatments	Haematological Parameters			
		Total WBCs count×10^3 cells/mm^3	RBCs (Million/mm)^2	Haemoglobin (g/dl)	ESR (mm/h)
1	Normal Control, 1% Tween 80, p.o.	8.18±0.90	7.88±0.11	14.82±0.13	11.40±0.42
2	Arthritic Control, 1% Tween 80, p.o.	14.70±1.12***	5.36±0.16**	10.46±0.12**	15.80±0.18***
3	Prednisolone 10 mg/kg, p.o.	8.48±0.75***	7.90±0.08**	15.20±0.31***	11.90±0.12***
4	Syringic acid, 50 mg/kg, p.o.	10.18±0.16**	6.24±0.18**	12.78±0.16**	13.40±0.16**
5	Syringic acid, 100 mg/kg, p.o.	9.26±0.31***	6.96±0.24*	13.48±0.45**	12.90±0.19***

Values are expressed as mean±SEM, *n*=6 in each group; *p<0.05, compared to disease control ** p<0.01, compared to disease control. ***p<0.001, compared to disease control

Table 5: Effects of Syringic acid on arthritic assessment in rats

S. No.	Groups & Treatments	Polyarthritic Index			
		7th Day	14th Day	28th Day	35th Day
1	Arthritic Control	3.31±0.12	3.56±0.18	3.96±0.20	4.30±0.16
2	Prednisolone 10 mg/kg	3.38±0.14	3.45±0.08	2.17±0.16**	1.50±0.18***
3	Syringic acid, 50 mg/kg	3.34±0.11	3.48±0.10	3.10±0.20*	2.20±0.21**
4	Syringic acid, 100 mg/kg	3.35±0.18	3.63±0.12	2.85±0.18**	1.85±0.16***

Values are expressed as mean±SEM, *n*=6 in each group; * p<0.05, compared to arthritic control**p <0.01, compared to arthritic control. *** p<0.001, compared to arthritic control

Estimation of biochemical parameters

Oxidative Stress Parameters

As shown in Table 6, MDA levels were observed to increase in Group II when compared with Group I. However, GSH levels and SOD activities were observed to decrease in Group II when compared with Group I. Administration of Syringic acid at dose of 100 mg/kg caused highly significant decrease (p<0.001) in MDA levels and increase in GSH and SOD activities.

Table 6: Effects of Syringic acid on oxidative parameters in rats

S. No.	Groups & Treatments	Oxidative stress parameters		
		MDA nmol/mg of protein	Glutathione µmol/g of protein	SOD U/mg of protein
1	Normal Control	4.50±0.21	7.48±0.26	7.85±0.34
2	Arthritic Control	14.25±0.51***	2.30±0.11***	3.10±0.11***
3	Prednisolone 10 mg/kg	7.50±0.25***	6.58±0.20***	5.85±0.20**
4	Syringic acid, 50 mg/kg	9.30±0.22**	5.40±0.22**	4.98±0.22*
5	Syringic acid, 100 mg/kg	8.10±0.24***	6.10±0.30***	5.78±0.21***

Values are expressed as mean±SEM, *n*=6 in each group; *p <0.05, compared to arthritic control** p <0.01, compared to arthritic control. *** p<0.001, compared to arthritic control

Membrane marker enzymes

A liver tissue was used to access the marked increase in the activity of membrane marker enzymes (ALP, LDH and ACP) in the arthritic rats when compared to control rats. There is significant increase in membrane marker enzymes of arthritic rats. Treatment with Syringic acid showed a moderately significant (p<0.01) decrease in the activity of membrane marker enzymes was seen in animals treated at 100 mg/kg.

Table 7: Effects of Syringic acid on membrane marker enzymes

S. No.	Groups & Treatments	Membrane Marker Enzymes		
		Alkaline Phosphatase (ALP) (µmoles of phenol formed/h/ mg protein)	Lactate Dehydrogenase (LDH) (µmoles of pyruvate liberated/min/ mg protein)	Acid Phosphatase (ACP) (×10−2 µmol of phenol formed/min/ mg protein)
1	Normal Control	0.42±0.02	8.43±0.11	2.20±0.16
2	Arthritic Control	0.91±0.08***	18.95±0.21***	6.32±0.10**
3	Prednisolone 10 mg/kg	0.50±0.04***	10.12±0.16***	2.80±0.12**
4	Syringic acid, 50 mg/kg	0.61±0.05**	12.44±0.21**	3.30±0.12*
5	Syringic acid, 100 mg/kg	0.53±0.01**	11.50±0.14***	2.90±0.16**

Values are expressed as mean±SEM, n=6 in each group; *p<0.05, compared to arthritic control ** p<0.01, compared to arthritic control. *** p<0.001, compared to arthritic control

Discussion

There is an emergent interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Thus, in the present investigation, an attempt was made to evaluate the anti-arthritic activity of Syringic acid on the basis of ayurveda and their traditional uses in a suitable experimental animal model.

CFA-induced experimental model for arthritis is considered closest to simulating human rheumatoid arthritis and therefore it is the most widely used chronic test model in which the associated clinical and Histopathological changes are comparable to those seen in human form (Billingham & Davies, 1979; Butler *et al.,* 1992) [3, 5].

The disease progresses rapidly over several weeks in what appears clinically to be a monophasic process. Granulocytes and auto reactive CD4 cells play major roles in the disease. Humoral immune mechanisms appear not to contribute to the disease process. This unique rat disease model represents a systemic process that involves not only the joints but also the gastrointestinal and genitourinary tracts, the skin and the eyes (Joe & wilder, 1999) [4].

The results emanating from the present study demonstrated that the Syringic acid dose dependently attenuated chronic inflammatory responses in adjuvant induced arthritis and also facilitated recovery as measured by the decreasing paw edema, body weight and various haematological parameters. The assessment of paw edema, an apparently simple, sensitive and quick procedure for evaluating the degree of inflammation in arthritic rats was done on alternate days in both the hind paws after adjuvant injection. Treatment with Syringic acid showed dose dependent suppression in edema of the injected paw (primary lesions). Maximal effects were observed at the dose of 100 mg/kg body weight. The suppression of this response therefore suggests any immunosuppressive activity for our Syringic acid. Moreover, this effect of Syringic acid was comparable to that of prednisolone.

During the development of arthritic syndrome, the body weight of rats used as an indirect index in restoration of health. Previous findings suggest that absorption of ^{14}C-glucose and ^{14}C-leucine in rat's intestine was reduced in the case of inflamed rats (Somasundaran *et al*., 1983) [23] but on the treatment with anti-inflammatory drugs, the decrease in absorption is neutralized. In our study, the body weight was significantly increased in the groups treated with prednisolone and Syringic acid treated groups and this may be due to the restoration of absorption capacity of intestine.

With the development of arthritic conditions, there was a significant alteration of haematological parameters i.e. red blood cells (RBCs), white blood cells (WBCs), Haemoglobin (Hb) and erythrocyte sedimentation rate (ESR). As the disease progressed, RBCs and haemoglobin were decreased whereas; WBCs and ESR were significantly increased in arthritic control group when treated with normal control.

The results of our study revealed that Syringic acid treated group's causes significant alterations in the hematological parameters and maximal effects were observed at 100 mg/kg. The reversal of RBC counts and Hb levels observed in case of Syringic acid treated groups could be attributed to the protective effects on tissue repair and suppression of disease progression. By modulation of immune system, syringic acid and prednisolone treated groups normalize the WBCs and ESR.

By comparing the results of the selected plants in FCA induced arthritis, it could be concluded that Syringic acid has most potent and highly significant anti-arthritic activity. Syringic acid significantly decreased paw volume, Polyarthritic index, body weight and generation of free radicals and lysosomal enzymes.

As the disease progresses, inflammation spreads to other organ like heart, liver and lungs and is identified by extra-articular characters like formation of nodules on extensor surface of the body. The nodules may also be developed in lungs. Connective tissue and blood vessels may also be damaged (Habermann & Cascino, 2006). According to severity, score is given (Zhang *et al*., 2009; Vogel, 2002) [28, 25].

An arthritic index for each animal is calculated as the sum of these scores. In our study, the average scores for each group of drug treated animals were compared with that of disease control animals. In disease control group, arthritic Index was significantly higher compared to normal control group while prednisolone and Syringic acid treated groups showed significantly less score as compared to model control group.

In arthritic animals, damage to the synovial cavity has been correlated with the overproduction of ROS, dysregulation of anti-oxidant enzymes and free radical-scavenging molecules in the joint (Sen, 1988, Cuzzocreo *et al.,* 2006) [7] and it is documented that in chronic and sub-acute inflammation, ROS plays an important role in modulating the extent of inflammatory response and consequent tissue and cell injury (Robbin *et al*., 2007) [20]. MDA is a metabolic product of lipid peroxidation, the level of which is raised in oxidative stress. Lipid peroxidation is a critical mechanism of the injury that occurs during rheumatoid arthritis, which is often measured by analysis of tissue Malonaldehyde. The large amount of Malonaldehyde in arthritic control group is consistent with the occurrence of damage mediated by free radicals. Syringic acid treated groups showed a significant attenuation of Malonaldehyde and having anti-

lipoperoxidative activity, which might be the proposed mechanism of anti-inflammatory activity of bioactive fractions in chronic inflammatory models. In our preliminary study, treatment of Syringic acid causes a significant decrease in WBCs and this might be due to the inhibition of lipid peroxidation and the consequent decrease in the chemotactic decrease of peroxide (Wills, 1987, Jira *et al*., 1997, Arulmozhi *et al*., 2011) [27,11, 2].

Decreased tissue glutathione has been associated with cell damage and depressed immunity. In arthritic control group, there was a significant decrease in glutathione levels in joints. Due to depletion of glutathione level, there is decreased protection against various invading pathogens (Talakal *et al*., 2000) [24]. Treatment of Syringic acid in treated groups causes a significant increase in glutathione level.

Superoxide dismutase (SOD) is a reactive enzyme that plays an important role in defense system of living cells. SOD inhibits the formation of superoxide-dependent chemotactic activity (Talakal *et al*., 2000) [24]. In arthritic animals, there was a significant decrease in SOD level and Syringic acid and standard treated groups showed a significant increase in this enzyme.

The significant up regulation of antioxidant enzymes, SOD, GSH and MDA by Syringic acid supports their role in anti-arthritic potential through effects on antioxidant parameters.

Hydrolytic enzymes are responsible for these changes which are stored in membrane bound cytoplasmic organelles i.e. Lysosomes. Lysosomes are widely distributed in tissues and circulating blood cells and are responsible for intracellular breakdown of complex macromolecules (Narendhirakannan *et al*., 2007) [12].

Increased activities of these enzymes were seen in liver (Olsen *et al*., 1990; Geetha, 1993) of arthritic rats. This may be attributed towards persistent inflammation. These changes are in agreement with the decreased lysosomal stability in adjuvant induced arthritis (Narendhirakannan *et al*., 2007) [12].

In our study, the activity of lysosomal enzymes were significantly increased in liver of arthritic rats and significantly reduced by treatments of Syringic acid. An important mechanism of anti-arthritic activity is the membrane stability modulating effect. The treatment of Syringic acid may exert its effects by modifying the lysosomal membrane in such a way that it is capable of fusing with the plasma membrane and thereby preventing the discharge of acid hydrolyses or by inhibiting the release of lysosomal enzymes (Chakraborthy, 2009) [6].

Conclusion

This study was designed to find out and prove the claims put forth by the traditional and folk medicine of applicability of Syringic acid. In conclusion, it can be stated that the Syringic acid have beneficial effects in long lasting reduction in rat paw edema, arthritic index and various lysosomal enzymes. It also showed a protective effect on arthritic rat joints without any toxic effect. The mechanism may be mediated via the inhibition of prostaglandin synthesis in acute inflammatory reaction as well as inhibition of various lysosomal enzymes in chronic inflammatory responses this, justifies the claim made by Siddha and Ayurveda.

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