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## Protective Effects of *Nephelium lappaceum* Rind Extract against Collagen-induced Arthritis in Dark Agouti Rats

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**Abstract:** Pathogenic mechanisms of arthritis are studied using Collagen-Induced Arthritis (CIA) animal models. Plant derived antioxidants are known to reduce the inflammatory response in CIA. The aim of the study was to assess the protective efficacy of *Nephelium lappaceum* ethanol extract against Collagen-Induced Arthritis (CIA) in dark agouti rats. Arthritis was induced with 4 mg kg<sup>-1</sup> of collagen in complete Freund's adjuvant. CIA rats were orally treated with 100 and 200 mg kg<sup>-1</sup> per oral of *N. lappaceum* from day 25-50. Changes in body weight, joint thickness, C-reactive protein were recorded and immunohistochemistry for matrix metalloproteinase-13 (MMP-13) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) was done. *N. lappaceum* (100 and 200 mg kg<sup>-1</sup>) significantly reduced (p<0.05) the arthritis-induced changes in body weight and paw edema. There was a significant reduction (p<0.05) in the C-reactive protein in the treatment groups. A significant reduction (p<0.05) in the arthritis-induced histopathological changes was seen after treatment with *N. lappaceum*. Treatment with *N. lappaceum* showed dose dependent effects on MMP-13 and TIMP-1 levels. *N. lappaceum* rind extract significantly suppressed the physiological, biochemical and histopathological changes produced during collagen-induced arthritis in dark Agouti rats. *N. lappaceum* extract supplementation may be beneficial in preventing the tissue damage and inflammatory conditions in arthritis.

**Key words:** *N. lappaceum* arthritis, collagen C-reactive protein, metalloproteinase, antioxidant, inflammatory condition

### INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic inflammatory disease that mainly targets the synovial tissue, cartilage and subchondral bone. Patients will suffer from joint inflammation, joint destruction and motor disability. Progressive and erosive destruction of the peripheral joints will lead to severe motor disability leading to decreased quality of life (Kumar *et al.*, 2009). The hallmark of RA is a persistent inflammation of synovial membrane, pannus formation and cartilage thinning which ultimately leads to joint deformities (Choy and Panayi, 2001; Navarro-Cano *et al.*, 2003).

Antioxidant micronutrients are presumed to play an important role in protecting against the tissue damage

caused by Reactive Oxygen Species (ROS). Antioxidants' provision in suppression of cytokines and collagenase expression induced by Tumor Necrosis Factor (TNF)- $\alpha$  has been demonstrated in RA patients (Halliwell *et al.*, 1988; Mahajan and Tandon, 2004; Sato *et al.*, 1996) which suggests additional mechanisms of protection against RA. The reports about the modulation of pro-inflammatory cytokine expressions by diet derived antioxidants have lead to the discussion that antioxidants have the potential to protect against rheumatoid arthritis. But there are very few researches available about the anti-inflammatory and antioxidant role of antioxidants from the plants in rheumatoid arthritis (Buchanan *et al.*, 1991; Mangee *et al.*, 1999; Panush, 1991; Shukla *et al.*, 2008).

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Rambutan (*Nephelium lappaceum*), a tropical fruit of the Sapindaceae family is native to Malaysia. This seasonal tropical fruit is an important commercial crop in Asia (Dembitsky *et al.*, 2011). The rind of *N. lappaceum*, habitually discarded, is having extremely high anti-oxidant (Palanisamy *et al.*, 2008; Thitilertdecha *et al.*, 2010), antibacterial (Martinez-Castellanos *et al.*, 2009; Thitilertdecha *et al.*, 2008), anti-hyperglycemic and free radical scavenging activities (Palanisamy *et al.*, 2011). Ethanolic *N. lappaceum* rind extract possess high free radical-scavenging activity and low pro-oxidant capability (Dembitsky *et al.*, 2011; Palanisamy *et al.*, 2011). The current study was designed to explore the anti-inflammatory effect of *N. lappaceum* rind extract on Collagen-Induced Arthritis (CIA) in rats. We hypothesize that the ethanol extract of *N. lappaceum* has significant anti-inflammatory effect and protects against arthritis-induced histopathological changes in Dark Agouti rats.

## MATERIALS AND METHODS

**Drugs and chemicals:** Type II collagen, Complete Freund's Adjuvant (CFA) and all other analytical chemicals used for the experiment were obtained from Sigma-Aldrich (St. Louis, MO, USA).

**Plant collection, isolation and standardization:** The extract was standardized using Geraniin as described previously (Palanisamy *et al.*, 2011). *N. lappaceum* was obtained from Kuala Lumpur, Malaysia from 2009-2010 and plants were authenticated by the herbarium of the Forest Research Institute of Malaysia (FRIM), Malaysia. Plants (1 kg) were cleaned and dried at 40°C in the oven and then, powdered using the Fritsch dry miller. Ethanol extraction (1:15; w/v) was carried out at room temperature in an orbital shaker. The suspension obtained was filtered using a 114 Whatman filter paper and filtrate was collected. Ethanol filtrate was concentrated using a rotary evaporator to yield a dark brown ethanol extract of rambutan rind.

**Determination of total phenolic content:** Total phenolics were determined based on a colorimetric oxidation and reduction reaction using the Folin-Ciocalteu method (Palanisamy *et al.*, 2008, 2011). Aliquot of the extracts (1 mL) was added to 5 mL of Folin-Ciocalteu reagent. After 3 min, 4 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution in water was added to the mixture and the content was thoroughly mixed. The absorbance at 765 nm was read after 1 h. Blank consisted of Folin-Ciocalteu reagent (5 mL), ethanol/distilled

water (1 mL) and 7.5% Na<sub>2</sub>CO<sub>3</sub> solution (4 mL). A linear dose-response regression curve was generated using absorbance reading of gallic acid at the wavelength of 765 nm. The calibration curve using gallic acid was obtained in the same manner as above except that the absorbance was read after 30 min. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW) of extracts (Palanisamy *et al.*, 2008).

**Experimental animals:** The autoimmune arthritis is mediated by sex hormones and is associated with a female preponderance for development of arthritis (Holmdahl, 1995; Van den Berg, 2009). Inbred female Dark Agouti (DA) rats of 6-8 weeks of age (200-220 g) were maintained at room temperature (24±2°C), with a 12 h light-dark cycle, relative humidity 60-70% and allowed food and water *ad libitum* (Tudave *et al.*, 2011).

**Research protocol approval:** All the experimental procedures were according to internationally approved ethical guidelines for the care of laboratory animals and the study got the approval from Institutional research and ethics committee.

**Collagen-induced arthritis (CIA) and drug administration:** Collagen-Induced Arthritis (CIA) in rats was induced as previously described, with minor modification (Brand *et al.*, 2007). Approximately 4 mg kg<sup>-1</sup> of type II collagen in complete Freund's adjuvant was administered intradermally in the tail, 2 cm distal to the base of the tail for each rat (Tudave *et al.*, 2011). On day 21 after the primary immunization, the rats were randomly divided into five groups (n = 6):

**Group 1:** Control

**Group 2:** Arthritis control

**Group 3:** 100 mg kg<sup>-1</sup> *N. lappaceum* extract

**Group 4:** 200 mg kg<sup>-1</sup> *N. lappaceum* extract

**Group 5:** 300 mg kg<sup>-1</sup> glucosamine

These rats were orally administered with *N. lappaceum* extract and glucosamine once a day from day 25-50.

**Body weight and paw thickness measurement:** The severity of arthritis was assessed by measuring changes in paw edema and joint thickness using a digital caliper. The body weight changes of the rats were also measured during the study period.

**Biochemical analysis:** All the rats were sacrificed 24 h after the last day of treatment. Approximately 5 mL of blood samples were collected by cardiac puncture. Blood was immediately centrifuged and plasma was separated and stored ( $-80^{\circ}\text{C}$ ) until further analysis. From the plasma, alanine transaminase (AST), creatinine, Blood Urea Nitrogen (BUN) and total protein assay was done using commercially available kits. C-Reactive Protein (CRP) levels in plasma were determined using Millipore® Rat C-Reactive Protein ELISA kit (CYT294) in accordance with the manufacturer's instructions. The CRP concentration of each of the samples calculated based on the standard curve obtained.

**Histopathological analysis:** Paw joints were collected at the end of experiment and fixed in 10% (v/v) neutral formalin. The joints were trimmed into 5-6 mm thickness, decalcified and the tissue was processed and embedded into paraffin blocks. The slides, stained with haematoxylin and eosin (H and E) were examined under a Nikon Eclipse 80 $\beta$  (CF160) bright field microscope.

**Immunohistochemistry for MMP-13 and TIMP-1:** The paraffin embedded sections were deparaffinized with xylene and rehydrated in a gradient of ethanol. Endogenous peroxidase activity was quenched with 0.5%  $\text{H}_2\text{O}_2$  in ethanol for 5 min. Immunohistochemistry was performed with the avidin-biotin peroxidase complex (ABC) technique. After being washed with Tris Buffer Saline (TBS), antigen retrieval was performed manually by using a hot water bath immersion of  $75^{\circ}\text{C}$  for an hour with Target retrieval solution (S1699, Dako) of pH 9.0 for 45 min. This lower-than-average temperature was used to prevent excessive destruction of the sample tissues (Lee *et al.*, 2008). The sections were blocked with peroxidase-blocking solution (S2023, Dako) at room temperature for 30 min and then incubated overnight at  $4^{\circ}\text{C}$  with the anti-TIMP1 (ab1827) and anti-MMP13 (ab3208) antibodies (Abcam), respectively. After washing with TBS three times, they were incubated with HRP-conjugated secondary antibodies (K0609, Dako) at  $37^{\circ}\text{C}$  for 30 min. After washing with TBS three times, all sections were visualized with diaminobenzidine (DAB) (K3467, Dako). The slides were counterstained with Mayer's hematoxylin and mounted with coverslip.

**Statistical analysis:** All the results were expressed as Mean $\pm$ SEM. Results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test (all pair wise multiple comparison procedure). A value of  $p < 0.05$  was considered statistically significant.

## RESULTS

**Changes in paw thickness:** The macroscopic sign of severe arthritis at 50th day included swelling, redness deformity and ankylosis in the hind paw and ankle joints. The symptoms of arthritic control rats showed significant difference ( $p < 0.05$ ) as compared to the hind paw of normal rats. The arthritic rats treated with *N. lappaceum* ( $100 \text{ mg kg}^{-1}$ ), *N. lappaceum* ( $200 \text{ mg kg}^{-1}$ ) and glucosamine ( $300 \text{ mg kg}^{-1}$ ) showed no significant changes in the joints when compared to arthritis alone group (Fig. 1). A significant increase ( $p < 0.05$ ) in the hind paw thickness was observed in the arthritic control rats from 0 to 50th day during the development of arthritis (Fig. 2). *N. lappaceum* treatment depicted a significant reduction in paw thickness compared to arthritic control at 50th day. The arthritic rats treated with *N. lappaceum* ( $200 \text{ mg kg}^{-1}$ ) also showed more significant reduction ( $p < 0.05$ ) in paw thickness during the development of arthritis as compared  $100 \text{ mg kg}^{-1}$  group (Fig. 2).

**Changes in the body weight:** Rats that developed arthritis showed reduction in body weight during first three weeks, found to be almost similar in all the groups of rats. However, after three weeks, the body weight of arthritic control rats was declined significantly ( $p < 0.05$ ) compared to its normal counterpart. *N. lappaceum* ( $100 \text{ mg kg}^{-1}$  and



Fig. 1(a-d): Hind-limbs distal inter-phalangeal joints of representative rat groups (a) Normal rat, (b) Arthritis (c) *N. lappaceum* treated  $200 \text{ mg kg}^{-1}$  and (d) *N. lappaceum* treated  $100 \text{ mg kg}^{-1}$

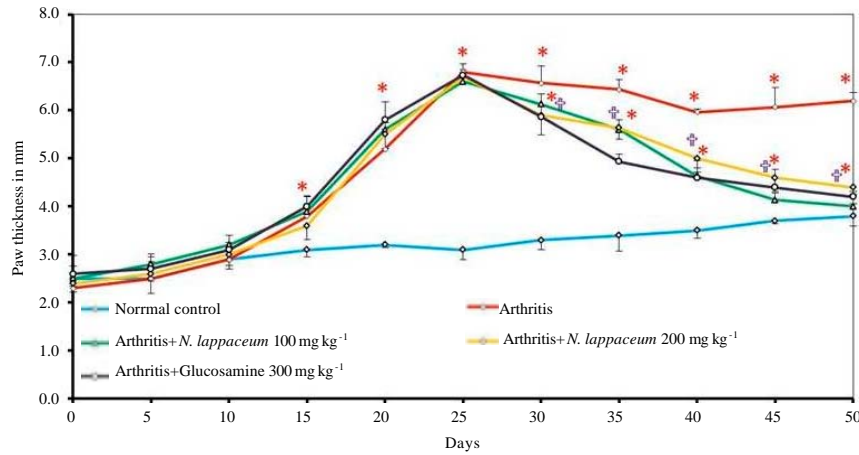


Fig. 2: Changes in paw thickness in normal, arthritis, *N. lappaceum* (100 and 200 mg kg<sup>-1</sup>) and glucosamine (300 mg kg<sup>-1</sup>) treated rats. Data are expressed as Mean±SE of six rats per group. \*p<0.05: Control vs. other groups, groups †p<0.05: Arthritis vs. other groups

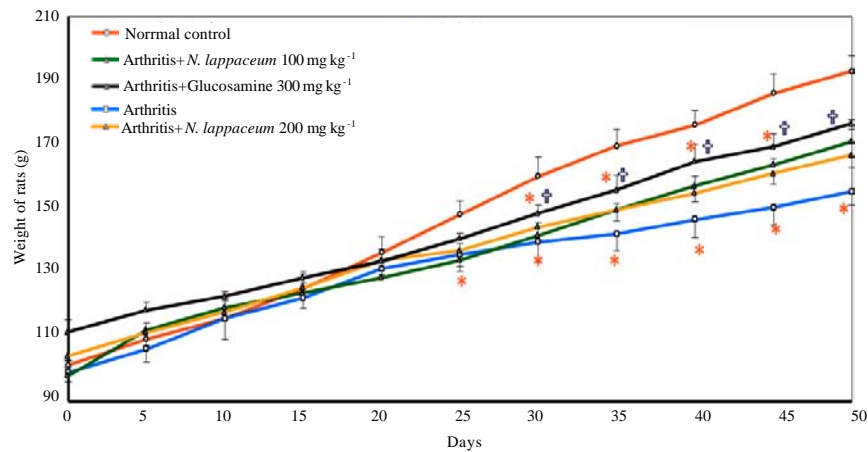


Fig. 3: Changes in body weight in normal, arthritis and *N. lappaceum* (100 and 200 mg kg<sup>-1</sup>) and glucosamine (300 mg kg<sup>-1</sup>) treated rats, data are expressed as Mean±SE of six rats per group, \*p<0.05: Control vs. other groups \*p<0.05: Arthritis vs. other groups

200 mg kg<sup>-1</sup>) treated arthritic rats showed a significant increment (p<0.05) in their body weight as compared to arthritic control (Fig. 3).

**Biochemical changes:** There were no significant differences in the AST, BUN, Creatinine and total proteins analysed between the controls and treated groups. There was a significant increase (p<0.05) in the CRP levels in the arthritis rats than controls. The concentration of C-reactive protein was found to be significantly reduced (p<0.05) in *N. lappaceum* treated as well as the glucosamine treated groups (Fig. 4). Glucosamine treatment group had shown more significant decrease in the CRP levels (p<0.05).

**Histopathological analysis of joints:** There was extensive proliferation of synovial cells, resulting in pannus formation and infiltration of mononuclear cells and neutrophils to the sub synovial region, with damage to the articular surfaces and discontinuity in the cartilage in arthritis rats. All groups except the baseline normal control showed varying levels of pathological changes (Fig. 5a). Severe arthritic and degenerative changes were observed, which showed reduction in higher dosage group (*N. lappaceum* 200 mg kg<sup>-1</sup>) (Fig. 5b, d). The degree of reduction of morphological changes was more in glucosamine (300 mg kg<sup>-1</sup>) treated group (Fig. 5e). There was healthy regenerative synovium and areas of fibrosis, angiogenesis and fibroblasts proliferation in the treatment groups.

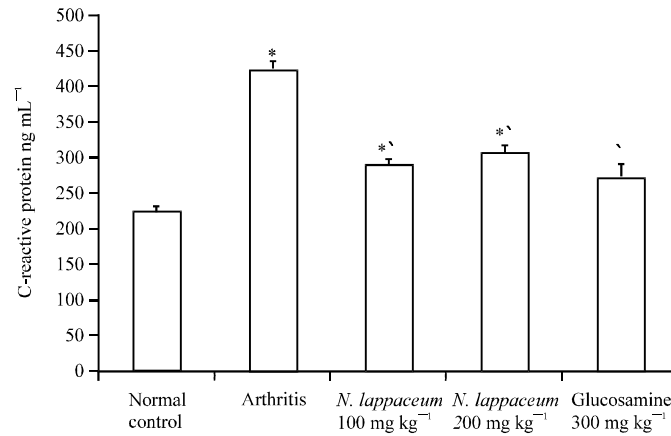


Fig. 4: Plasma C-Reactive Protein (CRP) levels in arthritis and treated groups, \* $p < 0.05$ : Control vs. other groups, <sup>^</sup> $p < 0.05$ : Arthritis vs. other groups

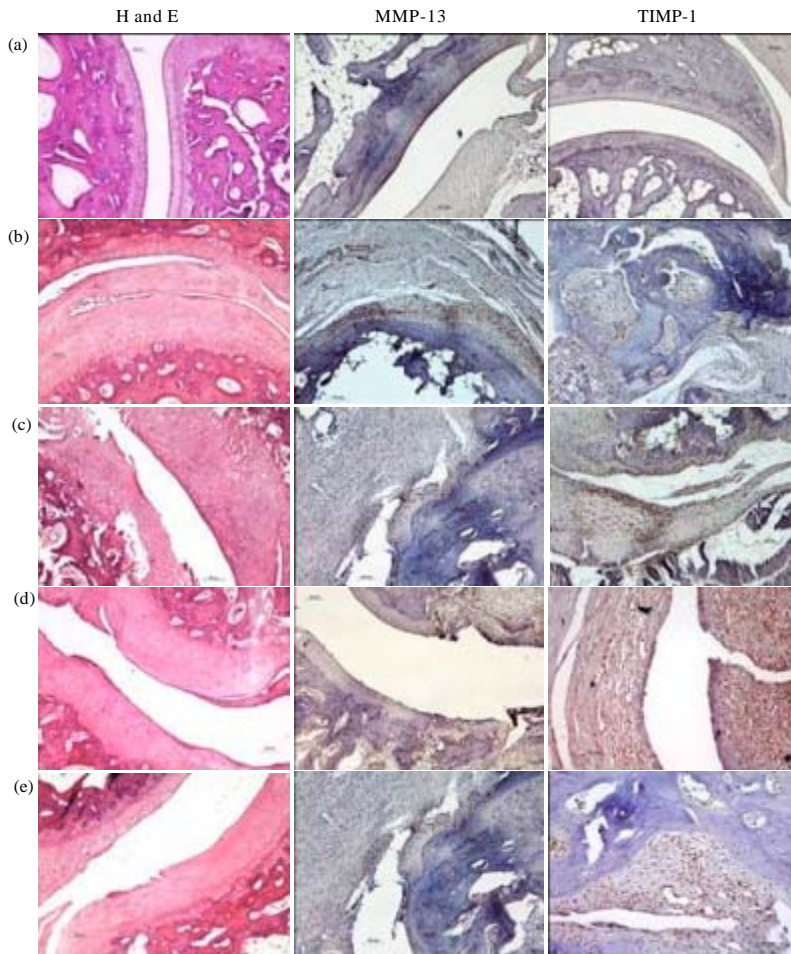


Fig. 5 (a-e): Histopathological analysis of joint morphology and immunohistochemistry for MMP-13 and TIMP-1 (200X) of (a) Normal control, (b) Arthritis, (c) *N. lappaceum* 100 mg kg<sup>-1</sup>, (d) *N. lappaceum* 200 mg kg<sup>-1</sup> and (e) Glucosamine 300 mg kg<sup>-1</sup> treated rats

### Immunohistochemistry of MMP-13 and TIMP-1:

Immunohistochemical expression of MMP-13 and TIMP-1 were observed in the sections under light microscopy and graded for positivity. IHC analysis indicated that the joint specimens from arthritic control rats strongly stained for MMP-13, which is a key contributor to the destruction of cartilage, however treatment with *N. lappaceum* reduced the expression of MMP-13 in inflammatory articular cartilage (Fig. 5c, d). It was seen that the groups having the worst histological findings, showed to have the highest levels of MMP-13 and the lowest levels of TIMP-1. Expression of MMP-13 was higher in the cartilage cells of the arthritic group and the least in *N. lappaceum* (200 mg kg<sup>-1</sup>) (Fig. 5c) and glucosamine (300 mg kg<sup>-1</sup>) (Fig. 5e). A corollary effect was seen for TIMP-1 (Fig. 5c), which showed the lowest expression in the above group.

### DISCUSSION

Classical signs of severe arthritis were observed with intradermal type II collagen administration, including symmetrical joint involvement typically involving the hind paws, swelling, redness and erythema over the joints (Asquith *et al.*, 2009; Brand *et al.*, 2007). The hallmark symptoms of RA include fever, fatigue and weight loss (Lee and Weinblatt, 2001). The body weights of CIA rats gradually decreases following immunization and it will be at the lowest after three weeks of immunization (Trentham *et al.*, 1977; Nagatomo *et al.*, 2010). Our study showed a significant loss in body weight around 25th day in the CIA rats as compared to the normal control. *N. lappaceum* (both doses) and glucosamine (300 mg kg<sup>-1</sup>) showed significant recovery in the body weight during the last 10 days of treatment when compared to their arthritic controls.

C-Reactive Protein (CRP) is produced in the liver under conditions of systemic inflammation in tissue destructions such as cartilage and bone and is a useful biomarker in the evaluation of disease progression and response to therapeutic intervention in a number of systemic inflammatory disorders, including RA. Higher concentration of CRP will indicate increased joint changes in arthritis (Poole *et al.*, 2008; Rhodes *et al.*, 2011). Severity of joint damage should correlate with CRP levels (Jones *et al.*, 2011). In our study, it is shown *N. lappaceum* exhibited a greater reversal of structural damage in the joints when compared with untreated arthritic rat. The levels of CRP quantified in our study showed that this correlation is true for the *N. lappaceum*, thus, proving the nutraceutical potential of the extract (Palanisamy *et al.*, 2008).

Histopathological changes correlated with macroscopic observations including changes in the paw thickness. Untreated arthritic rats showed maximum degenerative changes and significant reductions in the joint changes were seen in the treated groups. Observed protective effect may be due to the inhibition of inflammatory mediators by the anti-inflammatory compounds in the extract (Asquith *et al.*, 2009). An ellagitannin have been described to inhibit these mediators, primarily TNF- $\alpha$ , IL-1 $\beta$  and IL-6 [24] which could possibly correlate to the attenuation of histopathological changes.

Matrix metalloproteinases-13 (MMP-13) is thought to increase in degenerative bone diseases such as RA whilst tissue inhibitor of matrix metalloproteinases (TIMP-1) has the opposite action and it is an important factor in maintaining the integrity of connective tissue (Uchida *et al.*, 2000). Our results in this study showed an inverse relationship between MMP-13 and TIMP-1. Rats fed with the both doses of *N. lappaceum* extract seemed to be the most effective in increasing TIMP-1 expression and suppressing MMP-13 expression. Controlling MMP activity as well as the production of pro-inflammatory cytokines is required for the effective treatment of arthritis (McInnes and Schett, 2007). This observation provides evidence to suggest the potential anti-inflammatory effect of *N. lappaceum* against collagen-induced arthritis. The significant decline in symptoms of arthritis, paw thickness, histological symptoms and normalization of body weight and biochemical parameters in *N. lappaceum* treated arthritic rats postulates the possible anti-inflammatory and anti-oxidant effect of *N. lappaceum* rind extract. These effects may be attributed to the scavenging action of geraniin, an ellagitannin, present in the extract against Reactive Oxygen Species (ROS) such as OH, RO and RO<sub>2</sub> (Hagfors *et al.*, 2003).

### CONCLUSION

Thus, the present study showed that *N. lappaceum* rind extract is able to diminish the physiological, histological and biochemical changes produced during collagen-induced arthritis in Dark Agouti rats. *N. lappaceum* could be regarded as one of the natural products having vast range of therapeutic effects against collagen-induced arthritis. Further studies at the biochemical and molecular levels on the effectiveness and mechanisms of actions of *N. lappaceum* are being carried out.

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## REFERENCES

- Asquith, D.L., A.M. Miller, I.B. McInnes and F.Y. Liew, 2009. Animal models of rheumatoid arthritis. *Eur. J. Immunol.*, 39: 2040-2044.
- Brand, D.D., K.A. Latham and E.F. Rosloniec, 2007. Collagen-induced arthritis. *Nat. Protoc.*, 2: 1269-1275.
- Buchanan, H.M., S.J. Preston, P.M. Brooks and W.W. Buchanan, 1991. Is diet important in rheumatoid arthritis? *Br. J. Rheumatol.*, 30: 125-134.
- Choy, E.H.S. and G.S. Panayi, 2001. Mechanisms of disease: Cytokine pathways and joint inflammation in *Rheumatoid arthritis*. *N. Eng. J. Med.*, 344: 907-916.
- Dembitsky, V.M., S. Poovarodom, H. Leontowicz, M. Leontowicz, S. Vearasilp, S. Trakhtenberg and S. Gorinstein, 2011. The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. *Food. Res. Int.*, 44: 1671-1701.
- Hagfors, L., P. Leanderson, L. Skoldstam, J. Andersson and G. Johansson, 2003. Antioxidant intake, plasma antioxidants and oxidative stress in a randomized, controlled, parallel, Mediterranean dietary intervention study on patients with rheumatoid arthritis. *Nutr. J.*, Vol. 2.
- Halliwell, B., J.R. Hoult and D.R. Blake, 1988. Oxidants, inflammation and anti-inflammatory drugs. *FASEB J.*, 2: 2867-2873.
- Holmdahl, R., 1995. Female preponderance for development of arthritis in rats is influenced by both sex chromosomes and sex steroids. *Scand. J. Immunol.*, 42: 104-109.
- Jones, N.R., M.A. Pegues, M.A. McCrory, S.W. Kerr and H. Jiang *et al.*, 2011. Collagen-induced arthritis is exacerbated in C-reactive protein-deficient mice. *Arthritis. Rheum.*, 63: 2641-2650.
- Kumar, N., S. Singh, N. Patro and I. Patro, 2009. Evaluation of protective efficacy of *Spirulina platensis* against collagen-induced arthritis in rats. *Inflammopharmacology*, 17: 181-190.
- Lee, D.M. and M.E. Weinblatt, 2001. Rheumatoid arthritis. *Lancet*, 358: 903-911.
- Lee, K.H., Y.S. Chen, J.P. Judson, S. Chakravarthi, Y.M. Sim and H.M. Er, 2008. The effect of water extracts of *Euphorbia hirta* on cartilage degeneration in arthritic rats. *Malays J. Pathol.*, 30: 95-102.
- Mahajan, A. and V.R. Tandon, 2004. Antioxidants and rheumatoid arthritis. *J. Ind. Rheumatol. Assoc.*, 12: 139-142.
- Mangge, H., J. Hermann and K. Schauenstein, 1999. Diet and rheumatoid arthritis-a review. *Scand. J. Rheumatol.*, 28: 201-209.
- Martinez-Castellanos, G., K. Shirai, C. Pelayo-Zaldivar, L.J. Perez-Flores and J.D. Sepulveda-Sanchez, 2009. Effect of *Lactobacillus plantarum* and chitosan in the reduction of browning of pericarp Rambutan (*Nephelium lappaceum*). *Food. Microbiol.*, 26: 444-449.
- McInnes, I.B. and G. Schett, 2007. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat. Rev. Immunol.*, 7: 429-442.
- Nagatomo, F., N. Gu, H. Fujino, T. Okiura, F. Morimatsu, I. Takeda and A. Ishihara, 2010. Effects of exposure to hyperbaric oxygen on oxidative stress in rats with type II collagen-induced arthritis. *Clin. Exp. Med.*, 10: 7-13.
- Navarro-Cano, G., I. del Rincon, S. Pogolian, J.F. Roldan and A. Escalante, 2003. Association of mortality with disease severity in rheumatoid arthritis, independent of comorbidity. *Arthritis. Rheum.*, 48: 2425-2433.
- Palanisamy, U., H.M. Cheng, T. Masilamani, T. Subramaniam, L.T. Ling and A.K. Radhakrishnan, 2008. Rind of the rambutan, *Nephelium lappaceum* a potential source of natural antioxidants. *Food Chem.*, 109: 54-63.
- Palanisamy, U.D., L.T. Ling, T. Manaharan and D. Appleton, 2011. Rapid isolation of gerramin from *Nephelium lappaceum* rind waste and its anti-hyperglycemic activity. *Food Chem.*, 127: 21-27.
- Panush, R.S., 1991. Does food cause or cure arthritis?. *Rheum. Dis. Clin. North Am.*, 17: 259-272.
- Poole, C.D., P. Conway, A. Reynolds and C.J. Currie, 2008. The association between C-reactive protein and the likelihood of progression to joint replacement in people with rheumatoid arthritis: A retrospective observational study. *BMC Musculoskeletal Disorders*, Vol. 9.
- Rhodes, B., B.G. Furnrohr and T.J. Vyse, 2011. C-reactive protein in rheumatology: Biology and genetics. *Nat. Rev. Rheumatol.*, 7: 282-289.
- Sato, M., T. Miyazaki, T. Nagaya, Y. Murata, N. Ida, K. Maeda and H. Seo, 1996. Antioxidants inhibit tumor necrosis factor-alpha mediated stimulation of interleukin-8, monocyte chemoattractant protein-1 and collagenase expression in cultured human synovial cells. *J. Rheumatol.*, 23: 432-438.



- Shukla, M., K. Gupta, Z. Rasheed, K.A. Khan and T.M. Haqqi, 2008. Consumption of hydrolyzable tannins-rich pomegranate extract suppresses inflammation and joint damage in rheumatoid arthritis. *Nutrition*, 24: 733-743.
- Thitilertdecha, N., A. Teerawutgulrag and N. Rakariyatham, 2008. Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts. *LWT-Food Sci. Technol.*, 41: 2029-2035.
- Thitilertdecha, N., A. Teerawutgulrag, J.D. Kilburn and N. Rakariyatham, 2010. Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities. *Molecules*, 15: 1453-1465.
- Trentham, D.E., A.S. Townes and A.H. Kang, 1977. Autoimmunity of type II collagen: An experimental model of arthritis. *J. Exp. Med.*, 146: 857-868.
- Tudave, D., A. Radhakrishnan, S. Chakravathi and N. Haleagrahara, 2011. Modulation of C-reactive protein and tumour necrosis factor- $\alpha$  in collagen-induced arthritis in dark Agouti rats: Impact of collagen concentration on severity of arthritis. *Inflamm. Res.*, 60: 897-907.
- Uchida, M., M. Shima, T. Shimoaka, A. Fujieda and K. Obara *et al.*, 2000. Regulation of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) by bone resorptive factors in osteoblastic cells. *J. Cell Physiol.*, 185: 207-214.
- Van den Berg, W.B., 2009. Lessons from animal models of arthritis over the past decade. *Arthritis Res. Ther.* Vol. 11.