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Antiarthritic Activity of *Cocos nucifera* Linn against Formaldehyde Induced Arthritic Rat Model

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ABSTRACT

Aim: The present study was aimed at investing the protective role of hydroalcoholic extract of *Cocos nucifera* Linn against formaldehyde induced arthritic rat model. **Methodology:** Arthritis was induced by 0.1ml of 2% v/v formaldehyde in normal saline into the sub planter surface of the left hind paw on day 1 and 3 of study period followed by treatment with standard drug Indomethacin suspended in distilled water (10 mg/kg b.wt. i.p.) and hydroalcoholic extract of *Cocos nucifera* L. (200 & 400 mg/ kg, b.wt. p.o.) for 10 days. Arthritis was assessed by measuring the changes in body weight, paw diameter, plasma biochemical and haematological parameters and observed for the radiological examination. **Results and Discussion:** Treatment with test extract showed a significant ($P<0.001$) restoration of altered parameters exhibiting protective effect with an increase in body weight, reversal of plasma biochemical parameters and haematological parameters as evidenced by the radiological observations. **Conclusion:** The above findings suggest that *Cocos nucifera* Linn comprises phytochemicals that are responsible for maintenance of synovial membrane and recovery of bone destruction against formaldehyde induced arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder that is associated with symmetrical, inflammatory polyarthritis that may produce progressive joint damage. It is one of the most common inflammatory disorders affecting approximately 0.5–1.0% of global adult population, with females being affected three times more than males.[1-3] NSAIDS, Disease-Modifying Anti-Rheumatic Drugs (DMARDS), Biologic Response Modifiers and Corticosteroids are the most commonly used drugs for the treatment of RA.[4-6] Despite the progress made in the treatment of disease, these treatments fail to produce long term benefits and produce serious adverse effects such as gastrointestinal ulcer, renal morbidity, cardiovascular complications, haematological toxicity and nephrotoxicity, which limit their utility in the treatment of the disease.[7,8] Besides their side effects the current treatment is also of high cost, so patients suffering from chronic arthritis are likely to seek alternative methods for symptomatic relief.

Cocos nucifera Linn (Family: Palmaceae) commonly referred as Coconut or Nariel. Its bark is smooth and grey, marked by ringed scars left by fallen leaf bases. Unlike some other plants, the palm tree does not have tap root hairs but has fibrous root system. The activities of the root include astringent, dentifrice; decoction of root promotes flow of urine and is used in the diseases of the uterus, bronchitis and dysentery. It has antihelminthic activity and anti bacterial agent, in treatment for urinary tract infections and also in some skin infection.[9-10] It also posses wound healing property.[11]

The present study was undertaken to investigate the protective role of hydroalcoholic extract of *Cocos nucifera* Linn (HACN) against formaldehyde induced arthritic rat model.

MATERIALS AND METHODS

Chemicals: Formaldehyde was procured from the SD fine chemical, Chennai. Indomethacin was purchased from the Gland Pharma, India. All the diagnostic kits were procured from the span diagnostics, Mumbai.

Collection, Authentication and Preparation of the extract

The roots of *Cocos nucifera* L. were collected and authenticated **(Voucher No. 2547)** by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V. University, Tirupati,

Andhra Pradesh, India. The roots were shade dried for 15 days and pulverised into coarse powder. The coarsely powdered root of *Cocos nucifera* L. was subjected to extraction with hydroalcoholic solvent (70%) water: ethanol by cold maceration in a narrow mouthed bottle for seven days with occasional shaking using wrist shaker. After completion of extraction, it was filtered and the solvent was removed by evaporation using rotary evaporator and then dried.[12] Preliminary phytochemical screening of HACN was carried out for the detection of the various bioactive constituents.[13] Thus obtained dried extract was powdered and used for the present investigation.

Experimental Procedure

Healthy adult male Wistar rats were used for the study. Rats were housed in polypropylene cages, maintained under standardized condition which means that 12-hour light/dark cycle, 24°C and 35 to 60% humidity and provided free access to pellet diet and purified drinking water *ad libtium*. The animals were provided of food for 24 hour before experimentation but allowed free access to water throughout the experimental period. Arthritis was induced by 0.1ml of 2% v/v formaldehyde in normal saline into the sub planter surface of the left hind paw on day 1 and 3 of study period.[14] Animals were divided into five groups containing six animals each. Group I served as vehicle control received 0.5 mL of normal saline p.o., Group II served as arthritic control; Group III served as standard control received Indomethacin suspended in distilled water (10 mg/kg b.wt. i.p.); Group IV & V served as test control received hydroalcoholic extract of *Cocos nucifera* L. (200 & 400 mg/ kg, b.wt. p.o.). The total administration of the standard and test drugs was carried out for 10 days.

During the study period, paw diameter was measured at 0^{th} day, 1^{st} day, 3^{rd} day, 5^{th} day, 7^{th} day and 10^{th} day by using vernier calipers at either side of the rat left hind paw. On 11^{th} day, changes in the body weights were measured, animals were anaesthetized using mild anaesthesia and blood was collected through retro-orbital route. The blood was centrifuged at 4000 rpm for 10 min to separate the plasma. The plasma was used for the estimation of biochemical parameters such as alanine transaminase,[15] aspartate transaminase,[15] and alkaline phosphatase.[16] The blood samples were used for the determination of RBC, WBC & Hb.[17] The Joints were examined for the radiological observations.

RESULTS

The Phytochemical screening of the hydroalcoholic extract of *Cocos nucifera* L. revealed the presence of Carbohydrates, Flavonoids, Glycosides, Tannins and Proteins.

There was decrease in the body weights in formaldehyde induced arthritic rats when compared to Normal control. The treatments showed appreciable increase in body weights in standard (Indomethacin 10 mg/kg, b.wt.) and test groups (200 & 400 mg/kg, b.wt.) when compared to arthritic control group rats.

Table No. 1: Effect of HACN on change in body weights in formaldehyde induced arthritic rats

Control	Mean body weight (gms)		Mean difference in body weight
	Before induction	After induction	
Normal	118.5 ± 3.34	133.3 ± 2.78	14.8 ± 0.54
Arthritic	113.3 ± 2.27	89.75 ± 2.78	23.55 ± 0.39
Standard	108.3 ± 2.69	105.3 ± 1.03	3 ± 1.69***
HACN-I	117.0 ± 2.79	96.25 ± 0.85	20.75 ± 1.94**
HACN-II	115.3 ± 3.35	102.5 ± 1.04	12.8 ± 2.31***

Values were expressed as Mean ± SEM (n=6). *P<0.05,**P<0.01,***P<0.001.as compared with arthritic control (One-way ANOVA followed by Dunnet's test).

Graph 1: Effect of HACN on Mean Body weight difference in Formaldehyde induced arthritic rat model

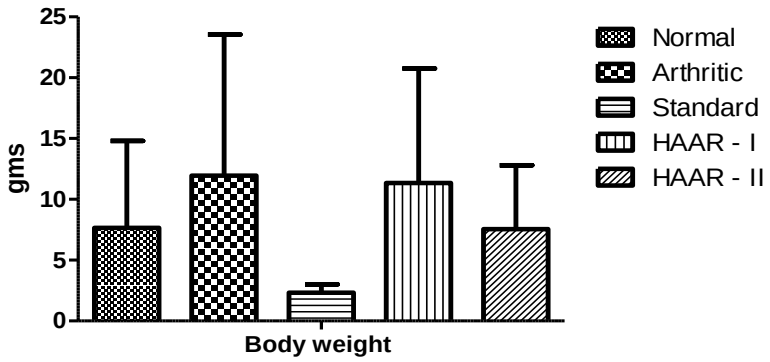

Figure No. 1: Effect of HACN on Mean Bodyweight difference in Formaldehyde induced arthritic rat model

A significant increase in paw diameter was observed that has reached to peak and remained constant by the end of 1st week in arthritis control as compared to vehicle control. Extract of

dose of 200mg/kg has shown moderate effect on prevention of paw edema, but the treatment with standard Indomethacin 10 mg/kg, b.wt. and extract dose of 400 mg/kg has shown significant prevention of paw edema as compared to arthritic control.

Table No. 2: Effect of HACN on paw diameter in Formaldehyde induced arthritis

GROUPS	PAW DIAMETER (mm)					
	Day 0	Day 1	Day 3	Day 5	Day 7	Day 10
Arthritic	9.1 ± 1.32	11.4 ± 1.31	14.8 ± 1.37	16.6 ± 1.59	17.6 ± 1.99	20.8 ± 1.42
Standard	9.6 ± 1.59	11.8 ± 1.72***	14.4 ± 1.59***	13.2 ± 1.32***	11.2 ± 1.42***	9.2 ± 1.32***
HACN -I	10.6 ± 1.59	12.9 ± 1.42***	15.8 ± 1.32***	14.7 ± 1.42***	12.4 ± 1.42***	10.4 ± 1.40***
HACN-II	10.6 ± 1.50	13.4 ± 1.99***	15.6 ± 1.40***	12.8 ± 1.72***	10.4 ± 1.59***	9.8 ± 1.77***

Values were expressed as Mean ± SEM (n=6). *P<0.05,**P<0.01,***P<0.001.as compared with arthritic control (One-way ANOVA followed by Dunnet's test).

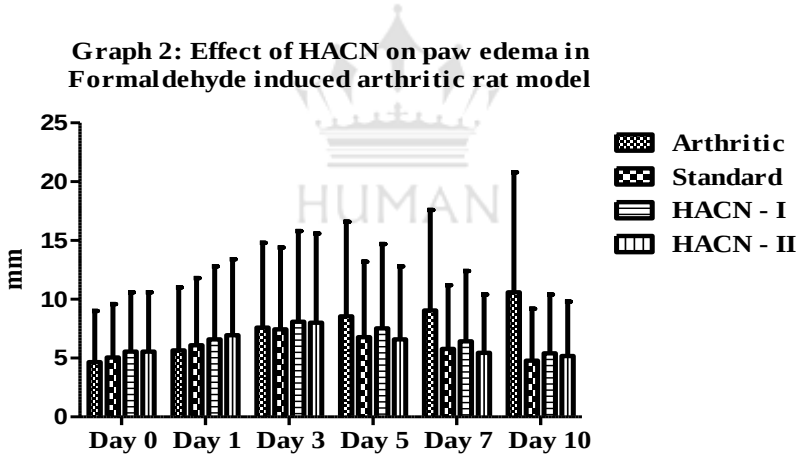

Figure No. 2: Effect of HACN on paw edema in Formaldehyde induced arthritic rat model

A significant increase in SGOT, SGPT, ALP and decrease in TP levels in formaldehyde induced arthritic control rats was observed. The Standard (Indomethacin 10 mg/kg, b.wt.) & HACN at both dose levels (200 & 400mg/kg, b.wt.) significantly restored the altered levels of SGOT, SGPT, ALP and TP levels when compared to arthritic control.

Table No. 3: Effect of HACN on serum biochemical parameters in Formaldehyde induced arthritic rats

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TP (g/dL)
Normal	42.65 ± 2.23	51.10 ± 2.34	143.2 ± 12.3	5.94 ± 1.04
Arthritic	93.97 ± 1.14	97.74 ± 2.09	343.7 ± 9.24	3.67 ± 0.97
Standard	58.90 ± 1.79***	55.76 ± 2.37***	164.5 ± 12.33***	6.91 ± 1.29***
HACN – I	72.91 ± 1.87***	87.72 ± 2.04***	241.4 ± 11.75***	4.91 ± 1.18***
HACN - II	67.25 ± 1.95***	60.63 ± 2.12***	182.3 ± 14.98***	5.71 ± 1.25***

Values were expressed as Mean ± SEM (n=6). *P<0.05,**P<0.01,***P<0.001.as compared with arthritic control (One-way ANOVA followed by Dunnet's test).

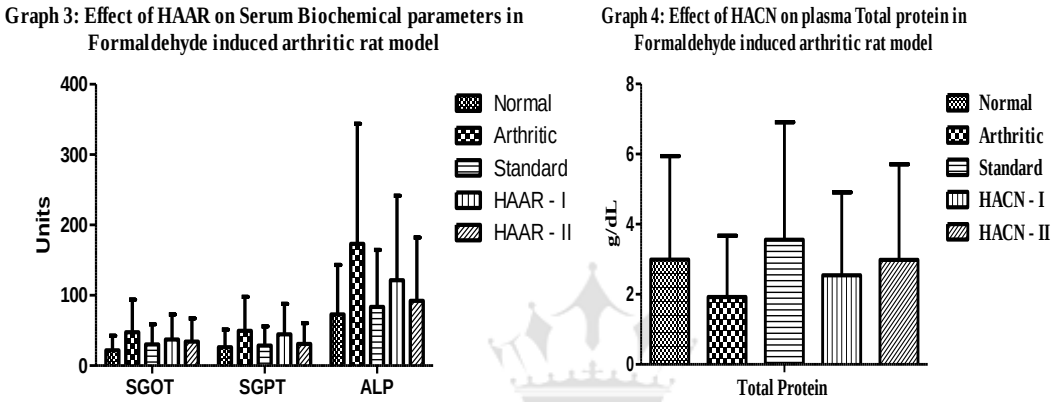

Figure No. 3: Effect of HACN on Serum Biochemical parameters in Formaldehyde induced arthritic rat model

Figure No. 4: Effect of HACN on plasma total protein in Formaldehyde induced arthritic rat model

Formaldehyde induction showed increase in WBC and decreased RBC & Hb count in arthritic control group as compared to vehicle control rats which was significantly ameliorated by test at both dose levels (200 & 400 mg/kg, b.wt.) and standard drug when compared to arthritic induced rats.

Table No. 4: Effect of HACN on haematological parameters in Formaldehyde induced arthritic rats

Groups	WBC (Cells / mm^3)	RBC (millions of cells / mm^3)	Haemoglobin (gm %)
Normal	7403 ± 3.22	5.97 ± 0.63	9.75 ± 0.43
Arthritic	13499 ± 4.27	4.10 ± 0.71	6.62 ± 0.31
Standard	6899 ± 4.27***	5.47 ± 0.73***	8.75 ± 0.43***
HACN – I	5199 ± 4.27***	4.74 ± 0.61**	7.45 ± 0.54**
HACN - II	6203 ± 3.22***	5.10 ± 0.85***	8.40 ± 0.64***

Values were expressed as Mean ± SEM (n=6). *P<0.05,**P<0.01,***P<0.001.as compared with arthritic control (One-way ANOVA followed by Dunnet's test).

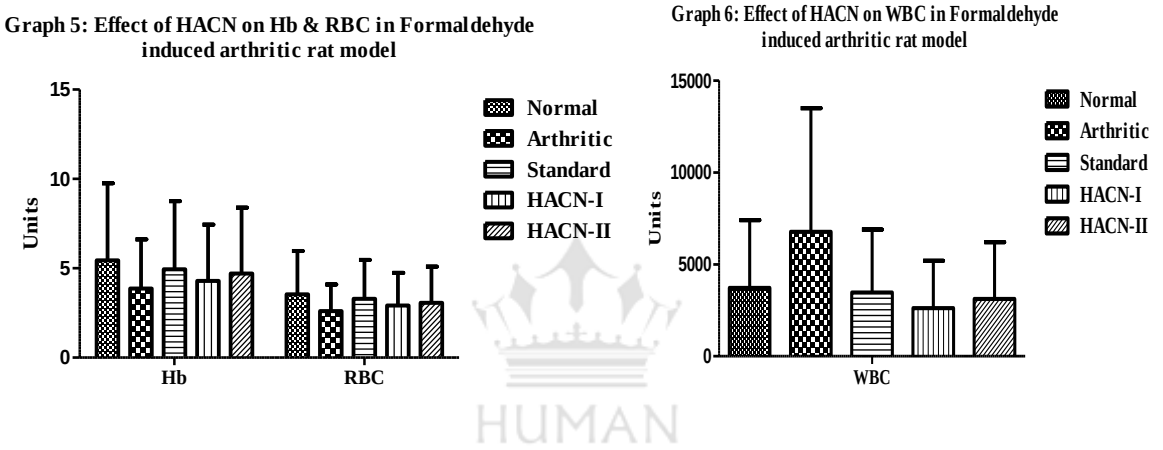

Figure No. 5: Effect of HACN on Hb & RBC in Formaldehyde induced arthritic rat model

Figure No. 6: Effect of HACN on WBC in Formaldehyde induced arthritic rat model

Radiographical Examination

Radiographic examination of Formaldehyde treated hind paw in arthritis control revealed several soft tissue swelling and narrowing of joint spaces as compared to vehicle control. Test extract doses of 200 mg/kg shown moderate effect on change in joint architecture. Treatment with standard Indomethacin (10 mg/kg, b.wt.) and extract dose of 400 mg/kg has shown considerable reduction in soft tissue swelling and narrowing of the joint space as compared to arthritis control.

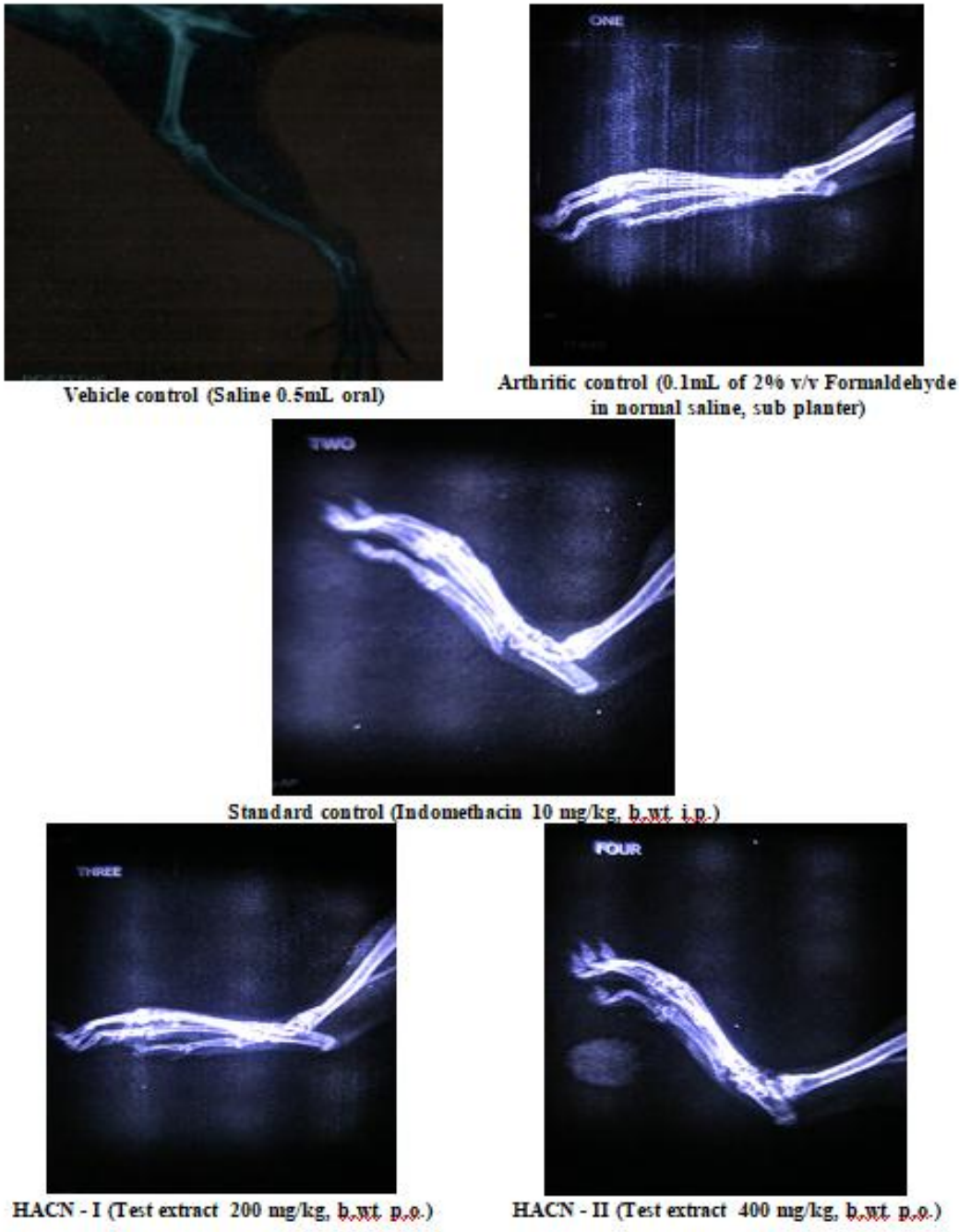

Figure No. 07: (A) Vehicle Control (B) Arthritic Control (C) Standard Control (D) HACN-I (E) HACN-II

DISCUSSION

RA is characterized by chronic inflammation and by the destruction of synovial joints, leading to joint deformity and disability. It is more common in females and affects around 0.5-1.0% of adults in the developed world. There are more than 100 forms of arthritis. Some of them are osteoarthritis, psoriatic arthritis and other related autoimmune diseases.[18]

The RA is caused due to the presence of pro-inflammatory markers, cytokines and leukotriene. The primary inflammatory markers are IL-1, TNF-α, IL-6, IL-15, IL-16, IL-17, IL-18, IFN-γ and granulocyte macrophage colony stimulating factor, chemokines such as IL-8, macrophage inflammatory protein-1 and monocyte chemoattractant protein-1. TNF-α blockade, IL-1 blockade, B cells therapy, IL-6 blockade and Angiogenesis blockade, these are therapeutic target for its treatment.[19]

In RA, destructive molecules are produced by an abnormal immune system response is responsible for continuous inflammation of the synovium. Collagen is gradually destroyed, with narrowing the joint space and finally damaging bone. In a progressive RA, destruction of the cartilage accelerates. Further pannus formation occurs due to the accumulation of fluid and immune system cells in the synovium that produces more enzymes destroys nearby cartilage, worsening the area and attracting more inflammatory white cells.[20]

The formaldehyde induced model of arthritis was useful to assess the potential anti-arthritic and anti-inflammatory effects of a substance partially resembles the characteristics of human arthritis.[21, 22] In the current study, arthritis was induced using 0.1ml of 2% v/v formaldehyde in normal saline into the sub planter region of the left hind paw, on day 1 and 3 of the study duration.[23] Formaldehyde induction causes chronic inflammation of the rat foot that involves the proliferation phase of inflammation elicited by COX mediators.[21, 22] Swelling around the ankle joint and paw of arthritic rat was considered to be due to the edema of particular tissue such as ligament and capsule.[24]

Formaldehyde induction elicits localized inflammation and pain in the early phase followed by subsequent phase of tissue mediated responses.[25] This late phase produces proliferative joint inflammation leading to articular changes similar to those seen in RA.[22] The development of edema in the paw of the rat after injection of formaldehyde is due to the release of histamine and prostaglandin like substances at the site of injection.[26] Prostaglandins are generated in primary inflammatory phase and autoantibodies are generated in secondary immunological state. Release of various inflammatory mediators including cytokines (IL-1B and TNF-α), interferons and PDGF are responsible for the initiation of pain along with swelling of the limbs and joints, bone deformations and disability of joint function.[27]

In the present study, significant weight loss in formaldehyde induced arthritic rats reporting rheumatoid cachexia leading to decreased physical activity, muscle strength and decreased daily performance was observed due to the altered metabolic activities.[28-30] Due to inflammatory condition, intestinal absorption of 14C-glucose and 14C-leucine was reduced resulting in decrease in body weights.[31,32] Significant increase in body weights with standard (Indomethacin 10 mg/kg, b.wt.) and test extract at both dose levels (200 & 400 mg/kg, b.wt.) might be due to restoration of the absorption capacity of intestines compared to the arthritic control group.

Formaldehyde induction significantly increased the diameters of rat paw and ankle as compared to the vehicle control due to soft tissue swelling around the ankle joints appeared during the progress of arthritis, considered as edema of the exacting tissues.[33] Treatment with standard (Indomethacin 10 mg/kg, b.wt.) and test extract at both dose levels (200 & 400 mg/kg, b.wt.) reduced the edema and paw edema as compared to the arthritic control that might be due to inhibition of the release of inflammatory mediators owing to its anti-inflammatory activity.

Elevated levels of plasma ALT and AST in formaldehyde induced arthritic rats can be due to increase in liver and bone fraction, implicates a localized bone loss in the form of bone erosion, as the enzymes are released into the circulation in the course of bone formation and resorption.[34] Increase in ALP in formaldehyde induced arthritic rats was due to disease causing bone remodelling causes its elevation.[35, 36] The treatment with the Standard and test extract significantly reduced the elevated levels of SGOT, SGPT & ALP as compared to the arthritic control.

Formaldehyde induces arthritis by denaturing proteins at the site of administration, producing immunological reaction against the degraded product results in decrease in plasma total protein levels.[37] The treatment with the test extract (200 & 400 mg/kg, b.wt.) and standard drug has shown significant increase in protein levels.

The rats with formaldehyde induction exhibited a reduced RBC and Hb level showing anemia as a common diagnostic feature with chronic arthritis.[38] Increase in WBC count is indications for the infectious and inflammatory diseases. Thus increased WBC can be attributed to systemic response of the rats to paw inflammation induced by formaldehyde.[39] The significant increase in Total WBCs count in disease control group may be due to the

stimulation of immune system against invading antigens.[40] The migration of leukocytes was significantly suppressed in rats treated with test extract and standard drug as observed from significant decrease in the WBC count.[41] The treatment with test extract and standard drug improved the RBC and Hb levels showing significant recovery from the anaemic condition.

Radiographic changes in RA condition were used as a diagnostic measure indicating the severity of the disease. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages of arthritis.[42] The radiographic features of the rat joints in formaldehyde induced arthritic model. In formaldehyde induced arthritic rats, soft tissue swelling along with narrowing of the joint spaces were observed which implies the bony destruction in arthritic condition. Treatment with standard anti-inflammatory drug and test extract prevented this bony destruction and swelling of the joint.

CONCLUSION

There is an increasing interest in herbal medications especially for chronic diseases like RA and plant remedies have become increasingly popular and are often preferred to synthetically derived pharmaceuticals. In the present study, formaldehyde induction in rats showed significant alterations in the body weights, paw diameter and plasma biochemical parameters and haematological parameters with radiological observations. Upon treatment with HACN at dose levels 200 & 400 mg/kg, b.wt. significantly ameliorated the abnormal plasma and haematological parameters supported by radiological examinations and comparison was made between the standard and arthritic control. From the above results, it was concluded that *Cocos nucifera* L. exhibited a prominent anti-arthritic effect imparting anti-inflammatory effect due to the presence of flavonoids and tannins contributed for the maintenance of synovial membrane, recovery of bone destruction thereby inhibiting cytokines and leukotriene infiltration inhibition.

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