Amelioration of Formaldehyde Induced Arthritis in Rats by *Solanum torvum* Sw. berries

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Abstract
Arthritis is a joint disorder featuring inflammation with over 100 types including osteoarthritis, rheumatoid arthritis, gout etc. The allopathic drugs used in the therapy carry the risk of side effects and arthritic drugs are no exception, the common side effects of anti-arthritic drugs are gastric ulcers, GIT bleeding, kidney & liver dysfunction, hypertension etc. The present study was aimed at diminishing the formaldehyde induced arthritis in rat model by hydro-alcoholic extract of Solanum torvum Sw. Arthritis was induced by administering 0.1ml of 2% v/v formaldehyde in normal saline into the sub planter region of the left hind paw on day 01 and day 03 respectively except vehicle control rats and grouped into five with six animals each. The total duration of the experimentation was carried out for a period of 11 days and groups were treated with standard (diclofenac sodium 10mg/kg, b.wt.) & hydroalcoholic extract of Solanum torvum Sw. (200 & 400 mg/kg, b.wt.) respectively. Formaldehyde induction in rats showed a significant alteration in the Physical parameters such as mean difference in the body weights, Paw diameter, hematological parameters RBC, WBC, Hemoglobin levels and plasma levels of SGOT, SGPT, ALP & Total proteins. Animals treated with the standard (diclofenac sodium 10mg/kg, b.wt.) & hydroalcoholic extract of Solanum torvum Sw. (200 & 400 mg/kg, b.wt.) significantly ($P<0.01$) ameliorated the altered levels promoting to its anti-arthritic activity.

Keywords: *Solanum torvum* Sw.*; Formaldehyde; Arthritis; Diclofenac Sodium.*

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1. Introduction

Hypersensitivity can manifest in different ways, leading to responses over human body cells and tissues, a condition known as autoimmune disorder [1]. Arthritis is a chronic, inflammatory and systemic autoimmune disease that affects the joints of human body affecting other tissues and organs such as heart, skin and muscles. Rheumatoid arthritis (RA) is an autoimmune and inflammatory illness disorder characterized by synovial polyarthritis with varying degrees of complexity in joints, extra articular and systemic signs that affect millions of individuals [2,3]. RA has been showing a global threat to many healthy individuals. However, 15% of the population i.e. 180 million people in India are nearly affected from RA [4] and the prevalence is boosting up than other diseases like cancer and diabetes. RA has reported to be ranked as second most common cause of disability and considered as a significant contributor to global disability burden [5]. The inflammatory synovium promotes the generation of a pannus, which is an abnormal layer of granulation tissue, causing progressive bone erosion and cartilage destruction [6]. The pro-inflammatory agent TNF-α secreted by the monocytes and macrophages in response to inflammatory agents, cachexia, and septic shock is a key cytokine of inflammation, which further manifests in tissue destruction [7, 8]. There is still no specific cure for rheumatoid arthritis, management of this condition depends on the use of non-steroidal anti-inflammatory drugs, Disease modifying anti rheumatic drugs and Corticosteroids but their long term usage has led to certain potential adverse reactions [9]. Therefore, to surpass all these issues and find a more harmless and equally efficacious therapeutic option, researchers are considering plants as a source of medicine. Herbal based therapies occupied the highest share of the international market, with annual turnover billion dollars in Western Europe as well as in China [10].

Solanum tovrum Sw. commonly known as ‘Turkey berry’ a member of the family Solanaceae is an erect spiny shrub of about 4mts tall evergreen and widely branched. It is native and cultivated in West Indies and Africa [11]. The fruits are utilized as a vegetable and regarded as an essential diet ingredient of most Africans and Indians commonly available in the markets [12]. However, *Solanum torvum* Sw. not only used as food but also widely used in traditional medicine in Africa and Asia for preventing and curing a wide range of health ailments [13, 14]. Various preparations of fruits, seeds or vegetative parts are indeed reported to be effective medicines against fever, cough, wounds, pain, liver troubles, tooth decay, reproductive problems, arterial hypertension and also as antidote in poisoning [15,16]. *Solanum torvum* Sw. properties include sedative, diuretic, hemostatic, haemopoietic, anti-microbial effects [13, 15, 17] as well as free radicals scavenging owing to its antioxidant activity [18]. Vitamins, essential organic nutrients which are necessary for smooth metabolism in the body, can also be found in *Solanum torvum* Sw. [19-23]. *Solanum torvum* Sw. as a promising natural source of phytochemicals has sufficient concentrations of alkaloids, flavonoids, Saponins, tannins, and glycosides exhibiting the medicinal properties, ranging from cardio-protection & treatment of heart related diseases, nephron-protection, to analgesic, anti-inflammatory, anti-ulcer and anti-microbial activities [17].

The present work was aimed at exploring the scientific validation of ethnopharmacological claim about *Solanum torvum* Sw. berries for its anti-arthritic activity.

2. Materials & Methodology

2.1 Collection, Identification and Authentication of plant material

The berries of *Solanum torvum* Sw. were collected from surrounding rural areas of Tirupati district and were authenticated by Dr. M.Niranjan Babu, Professor, Department of Pharmacognosy, Seven Hills College of Pharmacy, Tirupati, Andhra Pradesh, India.

2.2 Preparation of extract

The berries were washed, cut into small pieces of 2-3cm and shade dried at room temperature for 15 days. The shade dried berries were powdered using a dry grinder into fine powder. The finely grounded powdered berries of *Solanum torvum* Sw. was soaked in 200ml of (70%) hydroalcoholic solvent in 1:4 ratio in a narrow mouthed bottle for seven days with occasional shaking by maceration. After completion of extraction, the extract was filtered using Whattman filter paper and the solvent was evaporated to dryness using rotary evaporator [24]. The hydroalcoholic extract of *Solanum torvum* Sw. (HAST) was used for pharmacological screening of Anti-arthritic activity [25].

2.3 Animal Husbandry

Healthy adult male wistar rats weighing 200-225gms were used for the present study. Rats were housed in poly propylene cages, maintained under standardized 12-hour light/dark cycle, with a temperature 24 ± 2°C and 35 to 60% humidity providing free access to pellet diet and purified drinking water *ad libitium*. The animals were fasted 24 hour prior to the induction of arthritis but allowed free access to water throughout the experimental period.

2.4 Drugs and Chemicals

Diclofenac Sodium was supplied from Glory Pharma India Chem Pvt. Ltd., India. Formaldehyde, purchased from Sigma Aldrich Chemical, India. Plasma kits were obtained from Span Diagnostics Ltd. India. All other chemicals used in the experimentation were of analytical grade procured from reputed Indian manufacturers.

2.5 Experimentation

Rats were induced arthritis by administering 0.1ml of 2% v/v formaldehyde in normal saline into the sub planter surface of the left hind paw [26, 27]. Animals were divided into five groups containing six animals each. Group I received 0.5mL of normal saline p.o. served as vehicle control. Group II received 0.1ml of 2% v/v formaldehyde in normal saline at sub planter region of left hind paw on Day 01 and 03 served as arthritic control. Group III received Diclofenac Sodium (10 mg/k, b.wt.) i.p. and 0.1ml of 2% v/v formaldehyde in normal saline at sub planter region of left hind paw on Day 01 & 03 served as standard control. Group IV received hydroalcoholic extract of *Solanum torvum* Sw. (200 mg/ kg, b.wt. p.o.) and 0.1ml of 2% v/v formaldehyde in normal saline at sub planter region of left hind paw on Day 01 & 03 served as HAST-I. Group V received hydroalcoholic extract of *Solanum torvum* Sw. (400 mg/ kg, b.wt. p.o.) and 0.1ml of 2% v/v formaldehyde in normal saline at

sub planter region of left hind paw on Day 01 & 03 served as HAST-II respectively. The total duration of the experimentation was carried out for 11 days.

Paw edema was measured on day 01, 03, 05, 07, 09 and day 11 by using digital vernier calipers. The mean difference in the body weights of the animals were measured on the initial day after induction and at the end of experimentation. On day 11, blood was withdrawn from each animal through retro-orbital route for the assessment of hematological parameters such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb) [28, 29]. Blood was centrifuged by using bench top centrifuge at 3000rpm for 10 minutes to separate plasma. The obtained Plasma samples were analyzed for Serum Glutamate Oxalate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP) & TP using commercially available kit biochemistry analyzer (Microlab 400).

2.6 Radiological Examination

At the end of experimentation, the rats were anesthetized with pentobarbital (3ml/100gms/b.wt.) via intraperitoneal injection and Digital X-ray Specimen 4000 Pro System was applied to observe the radiological changes in formaldehyde induced rats (33 kV, 150_A, 8.9 ms). Images were read independently in a blinded fashion [30].

2.7 Statistical Analysis

The results were expressed as the mean ± SEM and analyzed using one way ANOVA followed by Dunnett`s comparison test with arthritic control. Data were computed for statistical analysis using the graph pad software (Prism 5.0 version).

3. Results

3.1 Effect of HAST on mean difference in body weights in formaldehyde induced arthritic rat model

The body weights in formaldehyde induced arthritic rats were significantly reduced compared to vehicle control. The control groups treated with standard (diclofenac sodium 10 mg/kg, b.wt.) and test (400 mg/kg, b.wt.) showed appreciable increase in body weights in when compared to arthritic control group while that of test at low dose (200 mg/kg, b.wt.) showed restoration of rheumatoid cachexia to near normal condition.

Table 1: Effect of HAST on mean difference in body weights in formaldehyde induced arthritic rat model

Control	Mean body weight (gms)		Mean difference in body weights
	Before induction	After induction	
Vehicle	218.5 ± 3.34	233.3 ± 2.78	(+)17.8 ± 0.56
Arthritic	213.3 ± 2.27	179.75 ± 2.78	(-) 33.55 ± 0.49
Standard	207.8 ± 2.69	219.3 ± 1.03	(+) 11.50 ± 1.66***
HAST-I	217.0 ± 2.79	212.25 ± 0.85	(-) 4.75 ± 1.94*
HAST-II	215.3 ± 3.35	219.5 ± 1.04	(+) 4.20 ± 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).

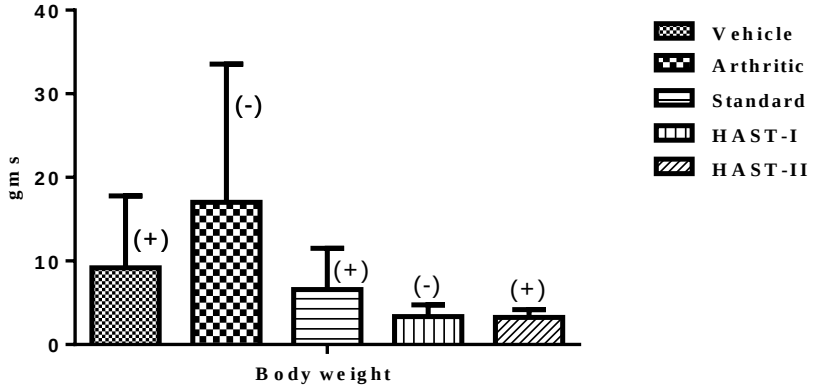


Figure 1: Effect of HAST on mean difference in body weights in Formaldehyde induced arthritic rat model

3.2 Effect of HAST on paw diameter in Formaldehyde induced arthritic rat model

Arthritic control showed significant increase in paw diameter which has reached to peak by the end of 1st week 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 diclofenac sodium 10 mg/kg, b.wt. while standard & test dose of 400mg/kg, b.wt. has shown significant reduction of paw edema.

Table 2: Effect of HAST on paw diameter in Formaldehyde induced arthritic rat model

Control	Paw Diameter (mm)					
	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Arthritic	3.81 ± 0.28	4.21 ± 0.34	5.14 ± 0.34	6.08 ± 0.23	7.74 ± 0.33	8.34 ± 0.28
Standard	3.74 ± 0.52	4.14 ± 0.58***	4.18 ± 0.38***	4.21 ± 0.18***	4.01 ± 0.17***	3.89 ± 0.14***
HAST-I	3.97 ± 0.41	4.08 ± 0.27*	4.88 ± 0.31*	4.82 ± 0.17*	4.71 ± 0.21*	4.62 ± 0.31*
HAST-II	3.53 ± 0.38	4.12 ± 0.28**	4.38 ± 0.18**	4.27 ± 0.21**	4.13 ± 0.13**	4.01 ± 0.21**

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).

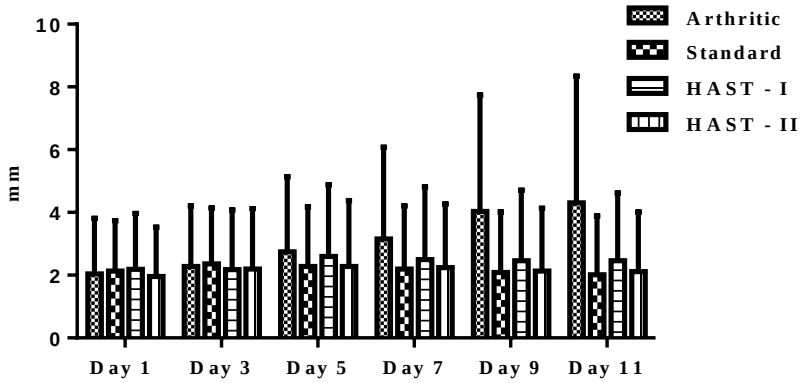


Figure 2: Effect of HAST on paw diameter in Formaldehyde induced arthritic rat model

3.3 Effect of HAST on plasma biochemical parameters in Formaldehyde induced arthritic rats

Formaldehyde induced arthritic rats showed significant elevation in SGOT, SGPT, ALP levels and decrease in TP levels. Treatment with standard (Diclofenac sodium 10 mg/kg, b.wt.) & HAST at both dose levels (200 & 400mg/kg, b.wt.) significantly restored the altered levels of SGOT, SGPT, ALP and TP levels compared to arthritic control.

Table 3: Effect of HAST on plasma biochemical parameters in Formaldehyde induced arthritic rats

Control	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TP (g/dL)
Vehicle	41.85 ± 2.23	50.10 ± 2.34	148.24 ± 12.3	5.62 ± 1.04
Arthritic	93.97 ± 1.14	97.74 ± 2.09	343.62 ± 9.24	3.67 ± 0.97
Standard	48.90 ± 1.79***	55.76 ± 2.37***	164.34 ± 12.33***	5.34 ± 1.29***
HAST-I	62.91 ± 1.87*	71.52 ± 2.04*	214.28 ± 11.75*	4.61 ± 1.18*
HAST-II	51.25 ± 1.95**	59.63 ± 2.12**	172.31 ± 14.98**	5.02 ± 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).

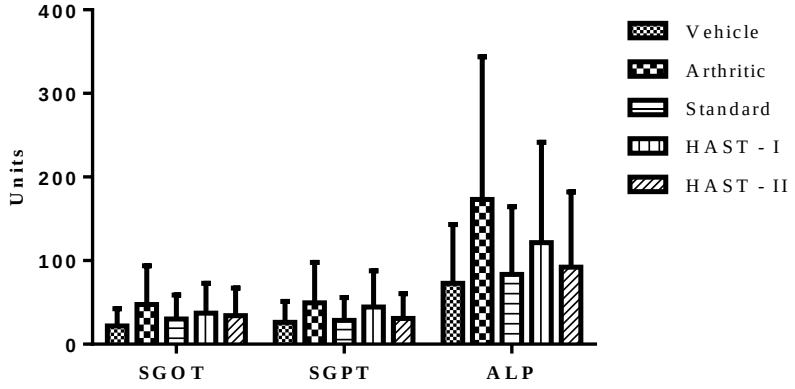


Figure 3: Effect of HAST on plasma biochemical parameters in Formaldehyde induced arthritic rats

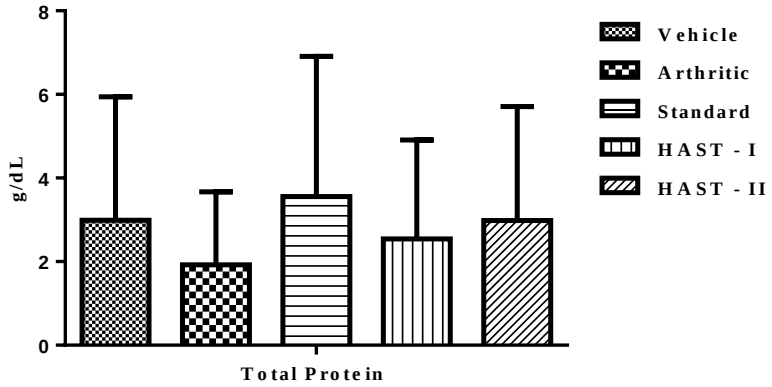


Figure 4: Effect of HAST on plasma total protein levels in Formaldehyde induced arthritic rats

3.4 Effect of HAST on haematological parameters in Formaldehyde induced arthritic rats

Formaldehyde induced arthritic control group showed increased WBC due to inflammation and invading antigens and decreased RBC & Hb count attributing to anaemic condition which was significantly restored by test at both dose levels (200 & 400 mg/kg, b.wt.) and diclofenac sodium.

Table 4: Effect of HAST on haematological parameters in Formaldehyde induced arthritic rats

Groups	WBC (Cells / mm^3)	RBC (millions of cells / mm^3)	Haemoglobin (gm %)
Vehicle	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***
HAST-I	5199 ± 4.27***	4.74 ± 0.61**	7.45 ± 0.54**
HAST-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).

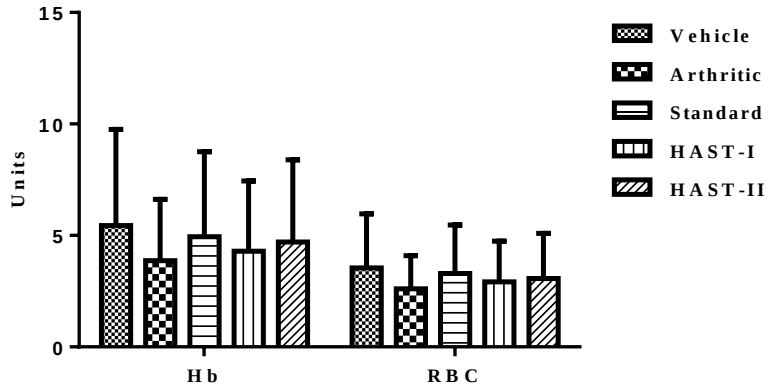


Figure 5: Effect of HAST on haematological parameters in Formaldehyde induced arthritic rats

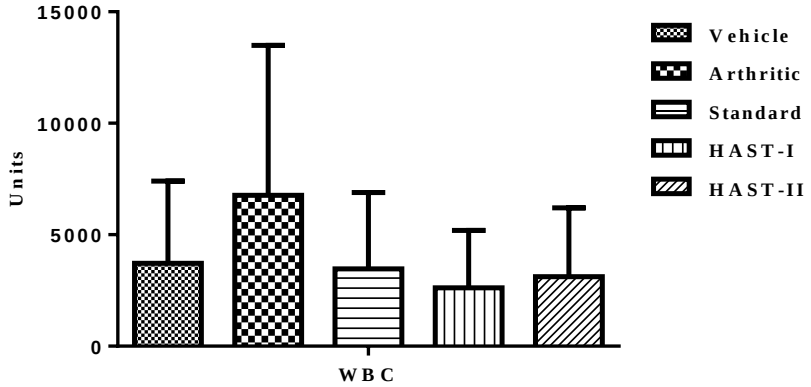
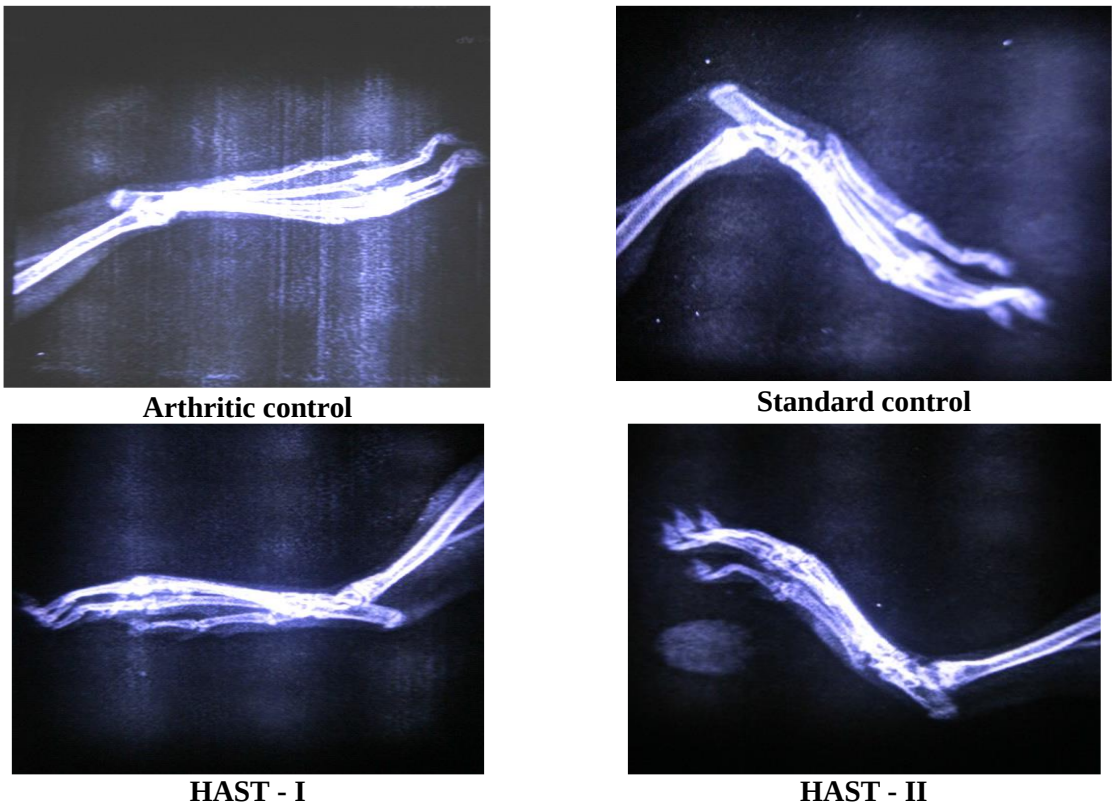


Figure 6: Effect of HAST on total WBC levels in Formaldehyde induced arthritic rats

3.5 Radiographic examination

Radiographic examination of Formaldehyde induced hind paw in arthritic control revealed swelling of soft tissue and narrowing of joint spaces. Test extract doses of 200mg/kg, b.wt. showed moderate effect on revert in joint architecture. Treatment with diclofenac sodium (10 mg/kg, b.wt.) and test dose of 400 mg/kg, b.wt. has shown considerable reduction in soft tissue swelling and narrowing of the joint space as compared to arthritic control.



Arthritic control **Standard control**

HAST - I **HAST - II**

Figure 7: Radiographical Examination of Synovial joints of formaldehyde induced arthritis in rats

4. 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 [31]. The formaldehyde induced arthritic rat model is very much beneficial to screen the potential anti-arthritic and antiinflammatory agents that partially resemble the characteristic features of human arthritis [32, 33]. In the current study, RA syndrome was induced using 0.1ml of 2% v/v formaldehyde in normal saline into the sub-planter region of the left hind paw, on the first and third day of the experimentation [32]. Formaldehyde administration develops a chronic inflammation in the rat foot, involving the proliferation phase of inflammation elicited by COX mediators [33, 34]. Swelling around the ankle joint and paw of arthritic induced rat is considered to be due to the edema of particular tissue such as ligament and capsule [35].

The early phase of the formaldehyde injection elicits localized inflammation and pain followed subsequently by a phase of tissue mediated responses [36]. The later phase produces proliferative joint inflammation leading to changes in the articular deformation [34]. The development of edema in the paw of the rat after formaldehyde administration is due to the release of histamines, serotonin and the prostaglandin like substances at the site of injection

[37]. Prostaglandins are generated in primary inflammatory phase and auto antibodies 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 joints, bone deformations and disability of joint function [38].

The weight loss in formaldehyde induced arthritic rats by altering the metabolic activity leads to rheumatoid cachexia causing decreased physical activity, muscle strength and decreased daily performance [39-41]. Arthritic Inflammatory condition reduces the intestinal absorption of 14C glucose and 14C leucine resulting in decreased body weights [42, 43]. Significant increase in body weights in animals treated with standard (Diclofenac Sodium 10 mg/kg, b.wt.) and test extract at high dose level (400 mg/kg, b.wt.) was observed attributing to restoration of the absorption capacity of intestine by reducing the inflammation.

The induction of formaldehyde significantly increased the diameters of rat paw and ankle as compared to the vehicle control due to the soft tissue swelling around the ankle joints appeared during the progress of arthritis, which was considered as edema of the exacting tissues [44]. Treatment with standard (Diclofenac Sodium 10 mg/kg, b.wt.) and test extract at both dose levels (200 & 400 mg/kg, b.wt.) reduced the paw edema compared to the arthritic control group may be due to inhibiting the release of inflammatory mediators owing to its anti-inflammatory activity.

Elevated levels of plasma SGPT and SGOT in formaldehyde induced arthritic rats can be due to increase in the liver and bone fraction, implicates a localized bone loss in the form of bone erosion, as the enzymes are released into the circulation during the course of bone formation and resorption [45, 46]. Increase in ALP in formaldehyde induced arthritic rats was due to disease causing bone remodeling promotes its elevation [47, 48]. The treatment with the standard and test extract significantly reduced the elevated levels of SGOT, SGPT & ALP compared to the arthritic control. Formaldehyde induces arthritis by denaturing protein at the site of administration, which produces immunological reaction against the degraded product [49] results in decrease in plasma total protein levels. Treatment with test extract (200 & 400 mg/kg, b.wt.) and standard has shown significant increase in protein levels.

The rats with formaldehyde induction exhibited a reduced RBC and Hb level causing anemia as a common diagnostic feature with chronic arthritis [50]. Increase in WBC count is the indications for the infectious and inflammatory condition attributed to systemic response of the rats to paw inflammation induced by formaldehyde [51]. The significant increase in total WBC count in disease control group may be due to the stimulation of immune system against the invading antigens [52]. The migration of leukocytes is significantly suppressed in test extract and standard treated rats as evident from the significant decrease in the WBC count [53]. The treatment with test extract and standard improved the RBC and Hb level showing significant recovery from the anemic condition.

A radiographic change in RA condition is useful diagnostic measure indicating the severity of the arthritis. 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 [54]. The radiographic features of the rat joints in formaldehyde induced arthritic model are shown in Figure 7. The soft tissue swelling along with narrowing of the joint spaces were observed in arthritic control rats implies the bony

destruction during arthritic condition. Treatments with standard drug and test extract groups have prevented the bony destruction and also reduced swelling of the joints was observed.

5. 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. It is therefore of interest to determine their active components and to elucidate their molecular mechanisms of action. The present study concludes that *Solanum torvum* Sw. is able to suppress the changes produced during formaldehyde induced arthritic condition in the rat models. However, further studies are needed to identify the mechanism(s) of action, involved in the anti-arthritic activity that would facilitate the use of *Solanum torvum* Sw. in Arthritic disorders.

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