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INVITRO AND *INVIVO* APPROACH OF LEAF JUICE OF *Raphanussativus* FOR ANTI-ARTHRITIC ACTIVITY

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ABSTRACT
Arthritis encompasses various autoimmune conditions characterized by joint inflammation, includes rheumatoid arthritis. There is a growing interest in natural remedies alongwith non-pharmacological interventions like hydrotherapy and acupuncture. *Raphanussativus* (radish, family: Brassicaceae) is recognized for its anti-arthritic properties and potential health benefits. This study aims to explore the anti-arthritic potential of *Raphanussativus* by *in vitro* and *in vivo* approach. *Raphanussativus* leaves were identified, collected, and authenticated, followed by prepared fresh leaf juice. Phytochemical tests were conducted to identify secondary metabolites in the leaf juice. The i*n vitro* pharmacological approach of fresh leaf juice of *Raphanussativus* (RSLJ) was done by Inhibition of protein denaturation method using BSA method and Inhibition of albumin denaturation by Egg Albumin Method. *In vivo* method involved the administration of RSLJ (200 and 400 mg/kg BW *P.O*) to rats with formaldehyde (0.1 ml, subplantar injection) induced arthritis for 14days, assessed parameters like body weight changes, haematological and biochemical parameters, and radiological alterations. The phytochemical analysis revealed the presence of alkaloids, glycosides, tannins, and flavonoids in RSLJ. The *in vitro* anti-arthritic activity of RSLJ was significant, with dose-dependent inhibition of protein denaturation comparable to diclofenac sodium. In FIA-induced arthritic rats, treatment with RSLJ demonstrated significant alterations in body weight, RBC, WBC, Hb%, ESR, C-reactive protein (CRP)Rheumatoid factor (RF) and uric acid. Radiological assessment showed attenuation of joint abnormalities with low and high doses of *Raphanussativus* leaf juice. The study highlights the potent anti-arthritic effects of RSLJ, attributing them to its phytochemical constituents such as alkaloids and flavonoids. Further research is warranted to elucidate its mechanisms and active constituents fully, and the leaf juice could be subjected to further isolation for identifying potent phytochemical constituents.
Keywords: *RaphanusSativus* leaf juice, arthritis, *In vitro*, *In vivo*, Formaldehyde.

1. INTRODUCTION:

Arthritis encompasses a spectrum of autoimmune disorder characterized by joint inflammation. Rheumatoid arthritis, a common form, causes severe joint pain as the immune system mistakenly attacks the body's tissues, potentially affecting multiple organs. In contrast, osteoarthritis results from the gradual breakdown of joint cartilage over time, leading to pain, stiffness, and limited mobility. Unlike rheumatoid arthritis, osteoarthritis is not autoimmune but rather a consequence of joint degeneration due to wear and tear. Gout arises from the accumulation of uric acid crystals in joints, triggering sudden and intense episodes of pain and inflammation, often affecting the big toe [1].

Various natural remedies are utilized to manage arthritis symptoms. *Aloe vera* is known for its anti-inflammatory properties and is often applied topically for joint pain relief[2]. *Boswellia,* extracted from the gum resin of the*Boswellia* tree, has anti-inflammatory effects and is used to reduce joint swelling and pain[3]. Cat's claw, sourced from *Uncariatomentosa* bark and root, exhibits anti-inflammatory properties and may help reduce joint discomfort and swelling[4]. *Eucalyptus*, derived from the leaves of the *eucalyptus* tree, contains compounds with analgesic and anti-inflammatory properties, commonly used in topical arthritis treatments. Ginger, known for its anti-inflammatory and antioxidant properties, may alleviate arthritis symptoms when consumed as a supplement or added to meals. Green tea, rich in polyphenol antioxidants, may help reduce inflammation and protect against cartilage damage in arthritis. Turmeric, containing curcumin, a potent anti-inflammatory compound, may help alleviate arthritis symptoms. Willow bark contains salicin, similar to aspirin, providing pain relief and anti-inflammatory benefits[5].

Raphanussativus, commonly known as radish and belonging to the Brassicaceae family, exhibits anti-arthritic properties. This herbaceous plant, widely consumed as a vegetable, is renowned for its rapid growth. In addition to these natural remedies, non-pharmacological interventions such as hydrotherapy, stretching exercises, massage therapy, and acupuncture are utilized to manage arthritis symptoms, improving joint function and mobility. These alternative approaches are effective in alleviating arthritis-related pain and inflammation, providing patients with additional options for pain relief and management. Radishes, appreciated globally for their crunchy texture and pungent taste, come in various colours including red, white, purple, and black. They are rich in vitamins C and K, as well as dietary fibre, making them a nutritious addition to a variety of dishes. Radishes are also recognized for their potential health benefits, including aiding digestion, promoting hydration, and supplying antioxidants to combat inflammation and oxidative stress. In addition to their culinary uses, radishes have a historical significance in herbal medicine for their diuretic, detoxifying, and digestive properties. They are often incorporated into traditional remedies and herbal teas for various ailments. *Raphanussativus* proves to be a versatile and nutritious vegetable with both culinary and potential medicinal applications [6, 7].

Raphanussativus, known as 'Radish seed' or Raphani semen, is a dried ripe seed widely utilized in traditional Chinese herbal medicine. In Telugu, it is termed as 'Mullangi.' Various extracts of *Raphanussativus* contain alkaloids, glycosides, saponins, tannins, carbohydrates, phenolic compounds, flavonoids, amino acids, and volatile oils. Nearly all parts of the plant, including leaves, seeds, and roots are utilized for medicinal purposes. While numerous medications are available for treating rheumatoid arthritis, there is a pressing need for developing alternative therapies, particularly herbal remedies that are safe and effective. *Raphanussativus* stands as a prime candidate from natural sources. Hence, our research aims to explore its potential as an anti-arthritic agent[8-10].

2. MATERIALS AND METHODS

Identification, Collection and Authentication of plant material

The leaves of *Raphanussativus* were identified and collected from near gardens of Venkataramapuram village, Tirupati district, Andhra Pradesh, India. The plant

Raphanussativus was authenticated by Dr. M. Niranjan Babu, Professor, Department of Pharmacognosy, Seven Hills College of Pharmacy (Autonomous), Tirupati, A.P, India.

Preparation of fresh leaf juice

The collected fresh leaves were well washed before being placed in a mortar and pestle and being ground into a fine paste. Then, using a coffee filter or cloth, pour the mixture through it to collect the filtrate (without adding any water)[11].

Phytochemical test

In order to determine the types of components and secondary metabolites present in plant leaf juice of the *Raphanussativus*, every day prepared leaf juice freshly. The initial qualitative phytochemical investigation was conducted using the recommended techniques fromHarbone, 1973[12], and Kokate, 2001[13].

The plant leaf juice *Raphanussativus* were estimated for secondary metabolites like alkaloids (Mayers test, wagers test, Hager's test, Dragendroff's test), Glycosides (Baljet test, Legals test, Keller-killani test), proteins and amino acids (Xanthoprotein test, Ninhydrine test), Flavonoids (Shinoda test, alkaline reagent test), Carbohydrates (Molisch's test, Benedict's test, Fehling's test, Barfoed's test), Tannins (Gelatin test), phenols (ferric chloride test), test for steroids and triterpenoids (LibermannBuchard test, Salkowski test), and Saponins (foam test, hemolytic test)[14-16].

2.1. *IN-VITRO* ANTI – ARTHRITIC ACTIVITY[17]:

Preparation of Bovine Serum Albumin Solution

A 5 % solution of Bovine serum albumin (BSA) solution was prepared by 5 gms ofBSApowderinwaterandmadeupto100mlinvolumetricflask.Thefinalconcentrationshouldbe50 mg/ml.

Preparation of Egg Albumin Solution

Theouter shell of theeggwas brokenwiththehelpof aglass rodandcolourless liquid albumin was separated in another beaker. And then was added to abeaker containing 100ml Nacl solution through constant stirring for 15 to 20 mins.Ensure that the solution is prepared well and egg albumin in water is formed. Nowfilter the content of the beaker with the help of a filter paper and the filtrate islabelledaseggalbuminsolution.

Principle:

Proteindenaturationisaprocessinwhichproteinslosetheirtertiarystructure andsecondarystructurebyapplicationofexternalstressofcompoundsuchasstrong acid or base, a concentrated inorganic salt, an organic solvent or heat. Mostbiologicalproteinslosetheirbiologicalfunctionwhendenatured.Denaturationofproteins is a well-documented cause of inflammation in RA. In this assay the proteinsdenaturation was measured and inhibition of protein denaturation was described asanti-arthriticactivity.

Inhibition of Protein denaturation method by BSA method

Procedure:

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5%aqueoussolution)and0.05mlofatdifferentconcentrationsofLeaf juice of *Raphanussativus*leavesat10,20,30,40,50µg/ml.Thenthesampleswereincubated at 37°C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3)was addedto eachtube andthe turbiditywas measuredspectrophotometric allyat660 nm. For blank control, 0.05 ml distilled water was used instead of extracts. Thecontrol represents 100%proteindenaturation. Theresults werethencomparedwithstanddiclofenac sodium(10µg/ml).

Thepercentageinhibitionofproteindenaturationwas calculatedbyusingtheformula

Percentageofinhibition(%) =(Acofcontrol-Asofleaf juice)/Acofcontrol×100

Ac= Theabsobance of blank control; As=Theabsorbanceofextracts

Inhibitionofalbumindenaturationby EggAlbuminMethod:

Procedure:

Thereactionmixturewas comprisedof0.2mlofeggalbumin,2.8mlofphosphatebufferedsalineofpH6.4and2mlofvaryingconcentrationof leaf juice of *Raphanussativus*10, 20, 30, 40, 50 µg/ml and similar volume ofdouble distilledwater was servedas control. Thenthe mixture was incubatedat 37°Cin incubator for about 15 mins and then heated at 70°C for 5 min. After cooling, theirabsorbance was measured at 660 nm by using blank. Diclofenac sodium (Standard drug)was usedas referencedrugandtreatedas suchfordeterminationofabsorbance.
Thepercentageinhibitionofproteindenaturationwas calculatedbyusingtheformula
Percentageofinhibition(%) =(Acofcontrol-Asof leaf juice)/Acofcontrol ×100

Ac= The absorbance of blank control; As=Theabsorbanceof leaf juice

2.2. *IN-VIVO* ANTI-ARTHRITIC ACTIVITY

Animals:

Male albino wistar rats (150-200g) were procured from the central animal house of Sri Venkateswara Enterprises, Bangalore. These were kept in the departmental animal house of Seven Hills College of Pharmacy (Autonomous) college, Tirupati at 23±2ºC and relative humidity 44-56%, light and dark cycles of 12 and 12h respectively for 1 week before and during the experiment for acclimatization.

After that, the studies were conducted and approved by the Institutional Animal Ethical Committee (IAEC) of Seven Hills College of Pharmacy (autonomous) College, Tirupati, Andhra Pradesh (Reg.no. 1521/po/a/11/CPCSEA). The standard gastric cannulas were used for oral drug administration in experimental animals.

Evaluation of Anti – Arthritic Activity:

Induction of arthritis by FIA[18];

Formaldehyde is used for induction of rheumatoid arthritis (RA) in rats and is currently considered as a model for reactive arthritis. Formaldehyde induced Arthritis (FIA): 2ml formaldehyde in 98ml of water makeup into 100ml (2% v/v). From that take 0.1 ml of formaldehyde and inject in the sub plantar region.On first day and 3rd day, they were injected into the sub plantar region of the right hind paw with 0.1ml2% v/v of Formaldehyde. Observe the paw volume after the injection of Formaldehyde.

Experimental design:

The animals were dosing with the test compounds & the standard was started on the same day and continued for 14 days.

Grouping of animals:

Group 1: Normal control-treated with saline 0.9% (for 14 days-*p.o*)

Group 2: Arthritic control (Formaldehyde0.1ml 2% v/v, Sub-plantar (SP) region on 1st and 3rd day only)

Group 3: Formaldehyde (1st and 3rd day) + Standard drug (Diclofenac sodium 10 mg/kg *i.p*. for 14 days)

Group 4: Formaldehyde (1st and 3rd day) + Low dose of *Raphanussativus*leaf juice (200 mg/kg b. wt. *p.o*. for 14 days)

Group 5: Formaldehyde(1st and 3rd day) + High dose of *Raphanussativus*leaf juice (400 mg/kg b. wt. *p.o*. for 14 days)

On day 1st, 7th and 14th evaluated body weights of the all groups of the animals. On the 14th day, the blood (up to 2ml) was collected by retro-orbital plexus puncture from each group 4 animals. The collected blood sample was used for analysis of haematological parameters and biochemical studies like RBC, WBC, Hb %, ESR, CRP, RF and uric acidrespectively[19].

On same day, from each group on animal were anesthetized with pentobarbital (3 ml per 100g body weight) by intraperitoneal injection, and Digital X-ray Specimen 4000 and 4000 pro System were applied to observe the radiological changes in Formaldehyde induced rats (33 KV, 150 A, 8.9 ms). The hind (arthritis induced) legs of the experimental rats were taken X-ray, and examined for the soft tissue swelling, bony erosions and narrowing of the spaces

between joints. Images were read independently in a blinded fashion and radiological score was recorded.

Statistical Analysis:

The results were presented as mean ± SEM, and statistical comparisons were conducted between the drug-treated group and the arthritic-control group. Statistical significance between two means was assessed using one-way ANOVA followed by Dunnett's multiple comparison test with In-Stat 3 statistical computer software. Mean values demonstrating statistical significance were those with $P<0.001$, $P<0.01$, and $P<0.05$, which were considered statistically significant.

3. RESULTS AND DISCUSSION

The preliminary phytochemical analysis of*Raphanussativus*leaf juice divulged that the following phytochemical constituents such as alkaloids, glycosides, tannins, and flavanoids.

3.1.Results- *In vivo anti-arthritic activity*

Effecton changesinbodyweight

The standard (Diclofenac sodium10mg/kg (*i.p.*)+ FIA (SP)and test drug (*Raphanussativus* 200mg/kg and 400mg/kg (*p.o.)* + FIA (SP) group rats exhibited a noticeable rise in body weight compared to the arthritic group (Formaldehyde0.1ml 2% v/v SP) rats, as illustrated in Table 1.

TableNo1:Effect of *Raphanussativus*leaf juice on body weight changes in FIAinducedarthritic rats:

Groups	Dose(mg/kg)	Bodyweight(gms) DAYS		
		Day1	Day7	Day14
I Normalcontrol	Normal Saline -0.9% *i.p.*	160±1.34	160±0.84	161±0.65
II Arthriticcontrol	Formaldehyde 0.1ml 2% v/vsubplantar (SP) region	155.20±1.13	151.40±2.40	146.25±0.91
III Standard control	Diclofenac sodium10mg/kg (*i.p.*)+ FIA (SP)	154.70±1.54	155.40±1.25	158.20±0.79
IV Test-I	*Raphanussativus* 200mg/kg (*p.o.)* + FIA (SP)	158.40±1.62	157.35±1.31	159.80±0.59
V Test-II	*Raphanussativus* 400mg/kg (*p.o.*)+ FIA (SP)	154.60±1.68	155.80±1.80	156.90±1.68

Valuesareexpressedasmean±SEM(n=5).p<0.05,p<0.01, p<0.001 Significantascomparedwitharthriticcontrol (One –wayANOVAfollowed byDunnet's test).

EffectonHematologicalparameters

The hematological parameters such as ESR and WBC count which was significantly decreasedby standard (Diclofenac sodium10mg/kg (*i.p.*)+ FIA (SP)and test drug (*Raphanussativus*200mg/kg and 400mg/kg (*p.o.)* + FIA (SP) group, whereas increased the levels of RBC count and hemoglobinin% in standard (Diclofenac sodium 10mg/kg (*i.p.*)+ FIA (SP)and test drug (*Raphanussativus*200mg/kg and 400mg/kg (*p.o.)* + FIA (SP) groupwhencomparedtoarthriticcontrolshownintable2 and 3.

TableNo2:Effectof*Raphanussativus*leaf juice onHematologicalparametersinFIA inducedarthriticrats

Groups	Dose(mg/kg)	Hematologicalparameters	
		Hb%	RBC

I Normalcontrol	Normal Saline -0.9% *i.p.*	12.43±0.40	8.17±0.069
II Arthriticcontrol	Formaldehyde 0.1ml 2% v/vsubplantar (SP) region	6.59±0.86	4.42±0.48
III Standard control	Diclofenac sodium10mg/kg (*i.p.*)+ FIA (SP)	11.68±0.70^{c}	5.48±0.28
IV Test-I	*Raphanussativus* 200mg/kg (*o.p.)* + FIA (SP)	10.00±0.70^{b}	4.39±0.57^{b}
V Test-II	*Raphanussativus* 400mg/kg (*o.p.*)+ FIA (SP)	11.82±0.50^{c}	6.31±0.67^{c}

Valuesareexpressedasmean±SEM(n=5).p<0.05,p<0.01, p<0.001significantascomparedwith arthriticcontrol (One –wayANOVA followed byDunnet's test).

Compared to arthritic control rats treated with FIA, the normal group rats treated with normal saline showed higher levels of HB. Additionally, when comparing the HB levels of FIA-treated rats to those receiving plant doses (200 mg/kg and 400 mg/kg LJRS plus FIA), the HB values were notably lower.

In comparison to the RBC levels of the normal group, the arthritic control rats treated with FIA displayed lower RBC counts. Furthermore, when examining the RBC levels of groups receiving plant doses (200 mg/kg and 400 mg/kg LJRS) in combination with FIA and standard treatment, it was evident that the FIA group had substantially reduced RBC counts, and similarly, the standard group (treated with Diclofenac sodium) also exhibited notably decreased RBC levels.

TableNo3:Effectof*Raphanussativus*leaf juice onHematologicalparameters (WBC and ESR)inFIA inducedarthriticrats

Groups	**Dose(mg/kg)**	**Hematologicalparameters**	
		WBC	**ESR**
I Normalcontrol	Normal Saline -0.9% *i.p.*	6.02±0.3	3.38±0.55
II Arthriticcontrol	Formaldehyde 0.1ml 2% v/vsubplantar (SP) region	9.98±0.74	8.64±0.74
III Standard control	Diclofenac sodium10mg/kg (*i.p.*)+ FIA (SP)	5.13±0.48	3.93±0.02
IV Test-I	*Raphanussativus* 200mg/kg (*o.p.)* + FIA (SP)	6.32±0.51	4.0±0.51
V Test-II	*Raphanussativus* 400mg/kg (*o.p.*)+ FIA (SP)	5.23±0.42	3.57±0.24

Valuesareexpressedasmean±SEM(n=5).p<0.05,p<0.01, p<0.001significantascomparedwith arthriticcontrol (One –wayANOVA followed byDunnet's test).

Compared to the arthritic control group treated with FIA, the normal group receiving normal saline exhibited lower WBC levels, as disease states typically entail gradual increases in WBC counts. Additionally, when comparing the WBC levels of groups treated with plant doses (200 mg/kg and 400 mg/kg LJRS) alongside FIA, higher WBC counts were observed in the FIA group. Furthermore, when comparing the standard treatment group with plant doses, a slight decrease in RBC levels was noted in the standard group.

Effecton CRP, Uric acidand RF

In arthritic control group treated with FIAshowed significant increases levels of CRP, Uric acid and RF as compared to vehiclecontrol (normal saline). The test 1 LJRS at a dose of 200mg/kg significantly decreased the levels of CRP, RF and uric acid.However standard control treated with diclofenac sodium and test 2 LJRS at a dose of 400mg/kg has shown significanteffect of preventing the elevated levels of CRP, RF and uric acid as compared to arthritic control (table no. 4 and 5).

TableNo4:Effectof*Raphanussativus* onCRPandRFin FIAinducedarthriticrats

Groups	**Dose(mg/kg)**	**CRP**	**RF**
I Normalcontrol	Normal Saline -0.9% *i.p.*	1.65±0.7	20.04±1.71
II Arthritic control	Formaldehyde 0.1ml 2% v/vsubplantar (SP) region	8.53±1.15	86.67±5.65
III Standard control	Diclofenac sodium10mg/kg (*i.p.*)+ FIA (SP)	3.06±0.76	82.59±2.65
IV Test-I	*Raphanussativus* 200mg/kg (*o.p.)* + FIA (SP)	3.42±0.75	20.36±1.48
V Test-II	*Raphanussativus* 400mg/kg (*o.p.*)+ FIA (SP)	2.57±0.91	20.28±3.80

Valuesareexpressedasmean±SEM(n=5).p<0.05,p<0.01, p<0.001significantascomparedwith arthriticcontrol (One –wayANOVA followed byDunnet's test).

Table 5: Effectof*Raphanussativus* on Uric Acidin FIAinducedarthriticrats

Groups	Dosemg/kg	Uric Acid
I Normalcontrol	Normal Saline -0.9% *i.p.*	239±0.7
II Arthriticcontrol	Formaldehyde 0.1ml 2% v/vsubplantar (SP) region	419±1.15
III Standard control	Diclofenac sodium10mg/kg (*i.p.*)+ FIA (SP)	218±0.76[c]

IV Test-I	*Raphanussativus* 200mg/kg (*o.p.)* + FIA (SP)	386±0.75[b]
V Test-II	*Raphanussativus* 400mg/kg (*o.p.*)+ FIA (SP)	225±0.91[b]

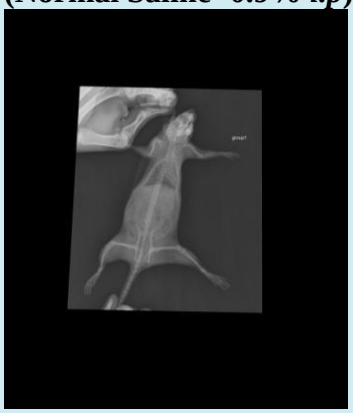
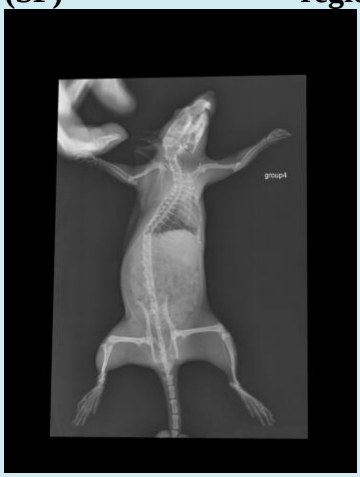
Valuesareexpressedasmean±SEM(n=5).p<0.05,p<0.01, p<0.001significantascomparedwith arthriticcontrol (One –wayANOVA followed byDunnet's test).

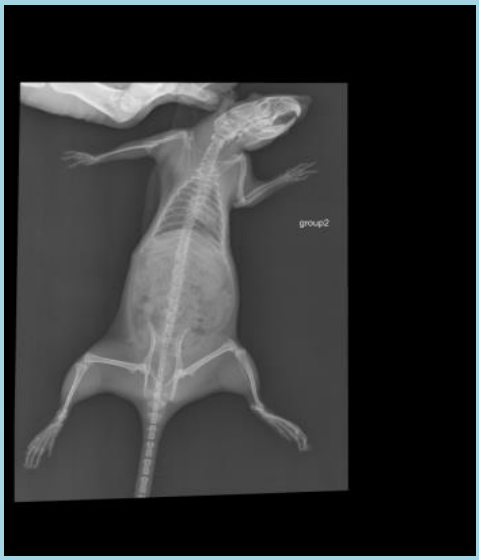
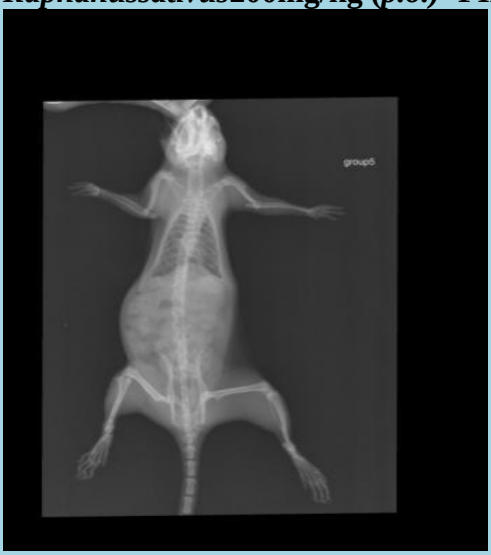
EffectonRadiologicalscoreassessment (Figure 1):

Rats administered with FIA developed noticeable joint abnormalities, including joint space narrowing in the inter-tarsal joints, widespread soft tissue swelling encompassing the digits, bone demineralization, significant periosteal thickening, cystic bone enlargement, and extensive erosions leading to joint space alterations. Conversely, rats treated with varying doses of 200mg/kg and 400mg/kg b.wt of *Raphanussativus* leaf juice exhibited mitigated abnormalities characterized by asymmetric soft tissue swelling, minor erosions, periosteal thickening, and limited joint space narrowing, primarily localized to the proximal areas of the paws.

Radiographic examination of FIA-treated hind paws in the arthritic control group revealed numerous soft tissue swellings and narrowed joint spaces compared to the vehicle control. Leaf juice at a dose of 200mg/kg exhibited a moderate effect on altering joint architecture. Treatment with standard diclofenac sodium and leaf juice at a dose of 400mg/kg demonstrated considerable reduction in soft tissue swelling and joint space narrowing compared to the arthritic control group.

Figure 1: Effect of *Raphanussativus* on Radiographicanalysis.

Group I: Normalcontrol **(Normal Saline -0.9% *i.p*).**	Group II: Arthriticcontrol **FIA Formaldehyde 0.1ml 2% v/vsub plantar region (SP)**	**Group III:**Standard control **Diclofenac sodium10mg/kg (*i.p.*)+ FIA (SP)**
		

Group IV: Test 1 ***Raphanussativus*200mg/kg (*p.o.)* + FIA (SP)**	**Group V:** Test 2 ***Raphanussativus*200mg/kg (*p.o.)*+FIA (SP)**
	

3.2. Discussion - *In Vivo* Anti-arthritic activity

Rheumatoid arthritis (RA) is a challenging autoimmune condition marked by persistent inflammation, joint damage, and systemic effects. The development of RA is influenced by a combination of genetic susceptibility and environmental factors, such as exposure to tobacco. Without intervention, RA advances steadily, causing substantial health complications and heightened mortality risks. Key symptoms include pain, swelling of joints, and morning stiffness, typically starting in small joints before affecting larger ones as the disease progresses[20-22].

While conventional management of RA primarily focuses on symptom relief and halting disease progression through pharmaceutical interventions, there is growing interest in the potential therapeutic benefits of natural plant extracts and compounds (NPECs) for RA treatment[48]. These compounds, derived from herbs and plants, exhibit a wide array of pharmacological activities, including immunomodulation and anti-inflammatory effects. Recent research has highlighted their potential to modulate the immune system and suppress pro-inflammatory cytokines. Given the multifaceted functions of NPEC medicines in RA treatment, exploring their potential holds promise for enhancing outcomes in RA patients. Additionally, radish juice, rich in urosolic acid, oleanolic acid, vitamin C, and various minerals, possesses notable anti-inflammatory and antioxidant properties, potentially beneficial for managing RA symptoms[23-30].

From the present study experimental period following induction with formalin-induced arthritis (FIA), noticeable changes in the body weights of the rats were observed. Previous studies indicate that fluctuations in body weight during arthritis progression could be linked to impaired absorption of glucose and leucine in the rat intestine. Reduced food intake due to limited mobility caused by hyperalgesia may also contribute to these changes. Treatment with different doses (200 and 400 mg/kg b.wt) of radish fresh leaf juice extract and Diclofenac sodium resulted in notable weight gain compared to the arthritic control group. This weight

gain suggests a potential restoration of intestinal absorption function, highlighting the therapeutic potential of radish leaf juice extract in mitigating arthritis-related alterations in body weight[31-34].

In this study, arthritic rats displayed decreased levels of red blood cells (RBC) and hemoglobin (Hb%), accompanied by elevated white blood cell (WBC) count and erythrocyte sedimentation rate (ESR). These findings collectively indicate an anaemic state, a common diagnostic feature in chronic arthritis patients. ESR levels are influenced by RBC number, size, and plasma protein concentrations, particularly fibrinogen and β globulins. Elevated ESR typically signifies on going but non-specific inflammatory processes. The acute phase proteins in ESR contribute to inflammation akin to responses seen in injections, injuries, surgery, or tissue necrosis. Treatment with radish leaf juice notably improved RBC count, Hb levels, and ESR, bringing them close to normal levels. This improvement suggests significant recovery from anaemia and arthritis progression, underscoring the potential therapeutic role of radish leaf juice in arthritic conditions[35].White blood cells (WBCs) are pivotal components of the body's immune defense system, crucial for responding to infections and inflammatory diseases. In arthritis, there is typically a mild to moderate increase in WBC count. Apart from prostaglandins, other products of cyclooxygenase and various cells involved in inflammatory responses, as well as free radical activities, contribute to the development of rat adjuvant arthritis. Radiographic evaluation of the knee joints in both arthritic and drug-treated animals provided additional evidence confirming the potent anti-arthritic effects in a dose-dependent manner[36-37].

In addition, rheumatoid factor (RF) serves as a primary serologic marker in arthritis, being an autoantibody that targets the Fc segment of IgG antibodies. The findings indicated that leaf juice positively influenced hematologic changes[38].

Furthermore, rats induced with formaldehyde-induced rheumatoid arthritis exhibited higher levels of uric acid (UA) compared to non-induced rats. Treatment with the plant leaf juice effectively managed the elevated UA levels, albeit to a lesser degree than the standard drug diclofenac[39-41].

Moreover, adjuvant disease is characterized by increased plasma levels of C-reactive protein (CRP), which closely correlate with the progression of the disease. CRP is synthesized by the liver in response to IL-6 during inflammatory conditions and plays a crucial role in antigen presentation. Treatment with standard diclofenac sodium and plant leaf juice (at a dose of 400 mg/kg) significantly lowered CRP levels. This reduction likely stems from the suppression of various stages of disease progression, highlighting the anti-rheumatoid arthritis activity of radish leaf juice extract. This activity may involve inhibiting arthritic processes, contributing to the observed effects on CRP levels[42-44].

In the current study, radiographic evaluations revealed the effects of different doses of *Raphanussativus* (radish) on experimental arthritis in rats. At a dose of 200 mg/kg body weight, mild inflammatory changes were observed in the synovial lining, while standard Diclofenac sodium maintained intact articular cartilage compared to the arthritis control group. Importantly, the higher dose of *Raphanussativus* at 400 mg/kg body weight significantly attenuated changes in joint architecture by reducing the presence of inflamed cells such as lymphocytes and eosinophils compared to the arthritis control group. Radiological analysis across all studies indicated a notable decrease or nearly completes absence of joint spaces in the hind paw bones of arthritis-induced rats.

3.3.Results and Discussion - *In vitro*Antiarthriticacativity

*In-vitro*antiarthriticactivityof leaf juice of *Raphanussativus*wascarriedoutusingBSAmethod.The effects of fresh leaf juice of *Raphanussativus* on inhibition of protein denaturationare shownintable 6;Figure 2. Juice of leaves at different concentrations (doselevels)providedsignificantprotectionagainstdenaturationofproteinsinadosedependentm

anner. Themaximum percentageinhibitionwas observedinfresh juiceof leaves about1.399at50 µg/ml. Itpossessedsignificantactivitycomparable to that of diclofenac sodium (50 µg/ml). $IC_{50}$of fresh leaf juice was found as 27.41µg/ml. From the results of present studyit can be stated that leaf juiceiscapableofcontrollingtheproductionofautoantigenandinhibitsdenaturationofproteininr heumaticdisease[45].

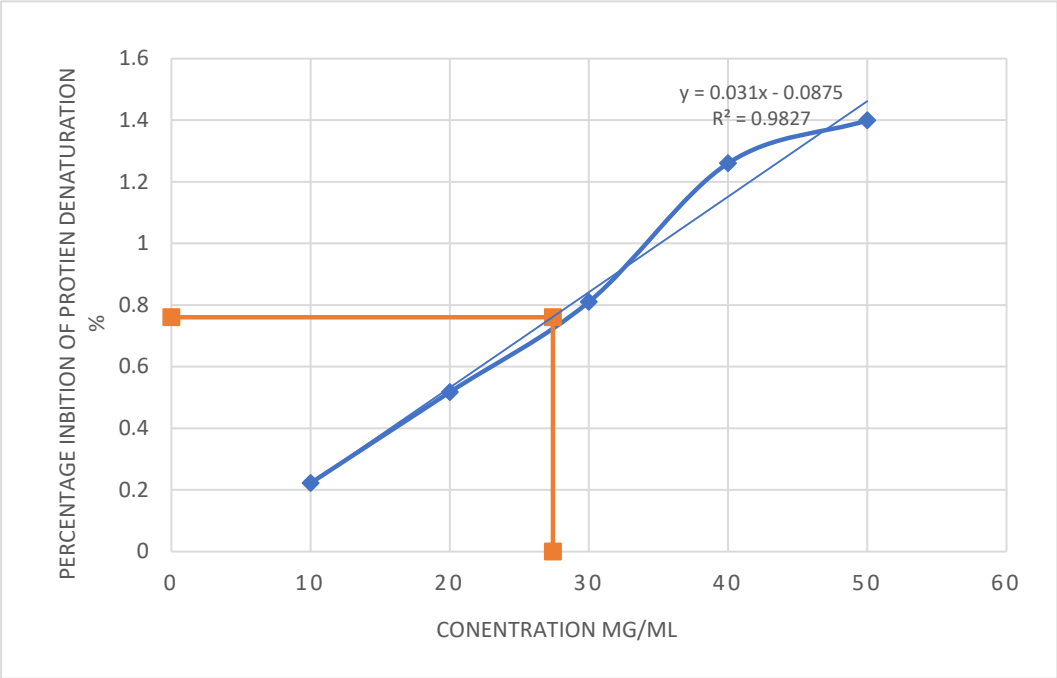
Medicinalplants used in traditional medicineto treatanti-arthriticconditions seem aviableandlogical alternativeinsearchof safeandeffectiveanti-arthriticagents[46]. *Raphanussativus*iscommonlyusedtraditionalmedicine in South Asian countries to treat inflammatoryconditions; hence, a simpleandviableproteindenaturationbioassaymethodwasselectedtoevaluateitspotential as anti-arthritic drug. Itis awellknownfactthatdenaturationof tissueproteins lead to inflammatoy and arthritic diseases[47]. Naturalproducts thatcanpreventproteindenaturationtherefore,wouldbeworthwhilefordevelopmentof anti-arthriticdrugtherapy.

Table6:*In vitro*anti-arthriticeffectof*Raphanussativus*byBSAmethod

Treatment	Concentíation (µg/ml)	PeícentageInhibition ofpíoteindenatuíation(%)
Raphanussativus leaf juice	10	0.222
	20	0.517
	30	0.810
	40	1.260
	50	1.399
	IC_{50}	27.41 µg/ml
Diclofenac sodium	50	22.90

Valuesaremean ± SD,n=3

Figure 2:*In-vitro*anti-arthriticeffectof*Raphanussativus* leaf juicebyBSAmethod



The effects of leaf juice of *Raphanussativus* on inhibition of albumindenaturationareshownintable7&Figure 3.Leaf juiceatdiffeíentconcentíations (doselevels) providedsignificantprotectionagainstdenaturationofproteinsinadosedependentmanner.Themaximumpercentageinhibitionwasobservedinjuice of leaves about1.499 %at50µg/ml. $IC_{50}$of leaf juicewas foundas 32.06 µg/ml. Itpossesses significantantiarthritic activitycomparableasthatofdiclofenac sodiumat 50µg/ml.

Table 7.*In-vitro*anti-arthritic effectof *Raphanussativus*leaves extractbyeggalbuminmethod

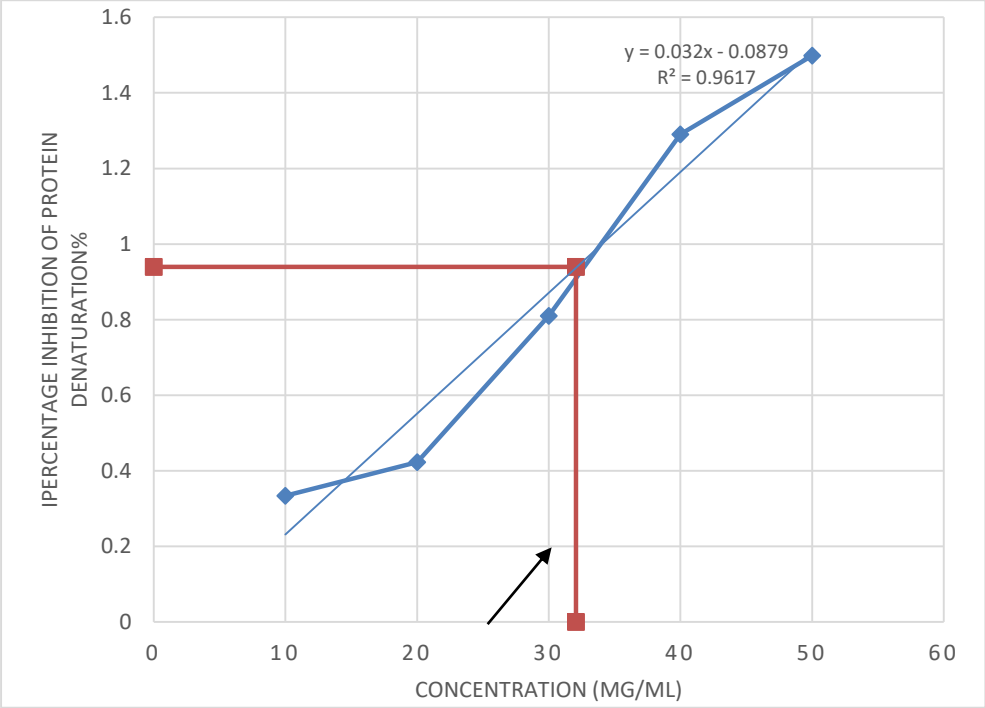
Treatment	Concentration (µg/ml)	PercentageInhibition ofprotein denaturation(%)
Raphanussativus leaf juice	10	0.334
	20	0.423
	30	0.810
	40	1.290
	50	1.499
	IC_{50}	32.06 µg/ml

Diclofenac sodium	50	26.32

Valuesaremean±SD,n=3.

The results of preliminaryphytochemical screening confirmed the presence ofvarious classes of secondarymetabolites in the *Raphanussativus*leaf juice includingpolyphenols (tannins andflavonoids).*Raphanussativus*leaf juiceat50mcg/mlandreferencedrugdiclofenac sodium (50mcg/ml)exhibiteddosedependentpercentageinhibitionofproteindenaturationinfresheggal buminandBSA.

Figure3:*In-vitro*anti-arthritic effectof *Raphanussativus*leaf juicebyeggalbuminmethod



6. CONCLUSION

Our study found that fresh leaf juice from *Raphanussativus* effectively mitigated formalin-induced arthritis in rats. In this model, secondary lesions like the arthritic index were assessed using evaluations of hematological, biochemical, and radiological parameters. Arthritic control rats displayed changes in body weight and elevated levels of biochemical markers such as RF, CRP, and uric acid. Hematological parameters including RBC count, WBC count, hemoglobin levels, and ESR were also analyzed in the arthritic control group. Treatment with *Raphanussativus* leaf juice restored joint integrity and normalized hematological and biochemical profiles compared to the arthritic control group.

Our findings indicate that *Raphanussativus* leaf juice exhibits significant in vitro anti-arthritic activity, supporting its traditional medicinal use. The study demonstrates the leaf juice's potential for arthritis treatment due to its substantial inhibition of protein denaturation.

The anti-arthritic effects of *Raphanussativus* leaf juice are attributed to its rich content of flavonoids, glycosides, alkaloids, tannins, and other bioactive compounds, as well as essential minerals including calcium, copper, potassium, vitamin C, folates, vitamin B6, manganese, and magnesium.

7. SCOPE FOR FURTHER STUDY

The current investigation focused on preliminary phytochemical analysis of *Raphanussativus*. Future research should include detailed chromatographic fingerprinting and chemical profiling of the plant, isolating various constituents and elucidating their chemical structures. This study also demonstrated the in vivo anti-arthritic activity of plant leaf juice from *Raphanussativus*. Further studies are needed to identify specific phytoconstituents responsible for this activity.

Moving forward, our research aims to elucidate the molecular mechanisms underlying the effects of the plant leaf juice. We plan to formulate herbal tablets using different combinations of the leaf juice and conduct in vitro evaluations to assess their pharmaceutical characteristics. Stability studies will be conducted to ensure the formulations' durability, and in vivo pharmacokinetic evaluations will assess their bioavailability. Finally, further clinical studies are necessary to validate these formulations for human use.

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