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Original article

Evaluation of Anti- inflammatory Effect of Pineapple Juice in Rheumatoid arthritis And Osteoarthritis Models in Rats

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ABSTRACT

Background: Arthritis is chronic disabling joint disease. Drugs available produce serious adverse effects on chronic use. Active Ingredient of pineapple fruit (Bromelain) appears promising adjuvant to anti-arthritic drugs. **Objective:** Evaluation of efficacy of pineapple juice in animal models of rheumatoid arthritis and osteoarthritis. **Materials and Methods:** First total 48 rats of either sex weighing 250-300 g were divided into 2 batches, (each containing 24 rats), batch I for osteoarthritis and batch II for rheumatoid arthritis . Each batch was subdivided in to 4 groups (n=6). Rheumatoid Arthritis induced by injecting 0.1ml Complete Freund's Adjuvant in sub plantar region of left hind paw. 24 Rats divided into 4 groups (n=6). Group-I: Control, Group-II: Pineapple juice, Group-III: Diclofenac, Group-IV: Pineapple juice + Diclofenac. Oral drug treatment was given from day0 to day12. Paw volume was measured by plethysmometer on day 0, 1, 5, 12, 21. Osteoarthritis induced by intra-articular injection of 50 µl Monosodium-Iodo-Acetate solution. 24 rats divided into 4 groups (n=6) as above and drug treatments given for 14 days. Animals were observed for joint inflammation and gait. On day 15 they were sacrificed, knee joints observed for gross changes and sent for histopathology. **Results:** Localized inflammatory reaction developed in all rats in 24 hours. In control group, no resolution of swelling, while in others, significant anti-inflammatory activity was seen which was maximum in combination Group-IV, followed by Diclofenac and least with Pineapple juice. Similarly significant anti-inflammatory activity was observed in terms of low histopathology scores in all 3 treatment groups of Osteoarthritis model. **Conclusion**: Results suggest that Pineapple juice has weak anti- inflammatory activity and can be added as a dietary adjuvant to standard anti-inflammatory drugs.

KEYWORDS: Pineapple, Rheumatoid arthritis, Osteoarthritis

INTRODUCTION

Arthritis is a chronic inflammatory disease. Individuals of any age can be affected with arthritis; however the usual age of onset is between 25-40 years which peaks in 40-50 years of age. Arthritis is associated with wearing down of the cartilages. The most commonly affected joints are weight bearing joints like feet, knees, hips, spine and other small joints such as fingers and thumb joints. Inflammation of the joint lining results in pain. Other symptoms include redness, swelling etc. All this can cause stiffness of the joints which can further result in loss of function [1]. The most common types of arthritis are osteoarthritis and rheumatoid arthritis. Several medications are currently used in the treatment of osteoarthritis. NSAIDs and other analgesics, corticosteroid injections and hyaluronic acid injections are used in the treatment of osteoarthritis.

However these treatments are riddled with several side effects like gastrointestinal ulcerations, gastrointestinal bleeding, fluid retention, congestive cardiac failures, and joint destruction are some of the side effects of these conventional therapies limiting their use [2]. Rheumatoid arthritis is treated with disease modifying anti-rheumatoid drugs like methotrexate, leflunomide, hydroxychloroquine and sulfasalazine. Other drugs used are immunosuppresants and TNFα inhibitors. These drugs are also associated with several limitations like immunosuppression, hepatic impairment, high cost of drugs, opportunistic infections etc. Hence there is a dire need for development of newer drugs, which are affordable and safe [2]. Pineapple is the common name of *Ananas comosus*. It belongs to the family Bromeliaceae and has been used in several countries for its medicinal properties.

The medicinal properties of pineapple have been attributed to bromelain. Bromelain is a crude extract from pineapple which contains several compounds like proteinases which exhibit activities like fibrinolysis, antithrombotic and anti-inflammatory [3]. It is used as a food supplement in the Europe and USA [4]. Also a case series reports the effect of bromelain in treatment of moderate to severe rheumatoid and osteoarthritis [5].

Several therapeutic benefits have been attributed to bromelain like inhibition of platelet aggregation, relief from sinusitis, treatment of thrombophlebitis, pyelonephritis, angina pectoris and bronchitis [4].Bromelain has been used in combination with trypsin and rutin and has been shown to be effective in treatment of osteoarthritis [6]. It has also been used as an alternative to NSAIDs [7]. However commercial preparations of bromelain are costly and long term use of these supplements are not cost effective. Hence the present study was undertaken to evaluate the effect of pineapple juice itself on animal models of osteoarthritis and rheumatoid arthritis since it not only contains Bromelain but also contains other beneficial ingredients like Vitamin C, calcium, manganese etc.

MATERIALS AND METHODS

Ethical Considerations

The study was conducted in accordance with the CPCSEA guidelines and was initiated after obtaining the approval of the IAEC

Animals

A total of 48 Wistar rats of either sex were used in the study. Six animals per cage were housed during the study period. Standard rodent feed purchased from Prashant Enterprises, Bhavani Peth, Pune, Maharashtra in form of pellets was provided. Aqua guard drinking water was provided ad libitum. As showed in table 1 rats were divided in two batches 24 each for two models of arthritis (one for rheumatoid arthritis and second for osteoarthritis).Each batch was further divided into four groups (n=6).

Table 1: Experimental groups for each model (Rheumatoid arthritis and osteoarthritis) (n=6)

Sr. No	Groups	Drug treatment
Group I	Vehicle control	Distilled water
Group II	Test drug	Pineapple juice treatment
Group III	Positive control	Diclofenac Sodium
Group IV	Combination group	Pineapple juice + Diclofenac

Animal models:

Rheumatoid arthritis model [8]: Evaluation of the anti-inflammatory activity was done by measurement of edema size resulting from Complete Freund's adjuvant injection in the left hind paw region. Normal paw volume and body weight were recorded on day 0. Induction of Inflammation was done with Complete Freund's adjuvant. The animals were anaesthetizes by 6 mg/100 gm, Pentobarbitone i.p., 0.1 ml of Complete Freund's adjuvant i.e. complete fraction of Mycobacterium tuberculosis suspended in mineral oil was injected in the sub-plantar tissue of the left hind paw. Treatment was started, as per groups on the same day and continued up to day 12 days. Body weight was measured on day 0, 1, 5, 12 and 21. Gait of all the animals was observed and scored according to, 0-Three legged gait, 0.5-Marked limping & 1-Normal gait. Paw volume was measured with Plethysmometer on day 0, 1, 5, 12 and 21. Paw volume in test group was compared with control and standard.

Osteoarthritis model [9]: Monoiodoacetate (MIA) was dissolved in saline 90 mg/ml. Under pentobarbital anesthesia, rat was positioned on the back and left leg flexed 90º at the knee. The patellar ligament was palpated below the patella and 50 µl (4.5 mg) of MIA solution was given by intraarticular injection through the patellar ligament of knee using 26G needle. Care was taken not to advance the needle too deep into the cruciate ligament.

Pineapple juice preparation- A ripe pineapple was taken; skin was peeled off and chopped. 100gms of chopped pineapple fruit was taken in a mixer, to which 20 ml water was added and ground. The mixture was filtered through stainless steel tea-strainer. Fresh pineapple juice was prepared every day. It was orally administered in the dose of 0.6 ml/ 100g body weight.

Diclofenac Sodium- Injection Voveran (Diclofenac Sodium 75mg/3ml i. e. 25 mg/ml) as purchased and was used as standard anti-inflammatory agent. Dose- 15mg/kg orally [23].

The treatments of the respective groups were stared on the same day and continued up to day 14. On day 15 rats were sacrificed; skin of the knee joint was removed and the joints were observed for gross changes. Knee joints were cut by bone cutter and fixed in 10% formalin. All joints were decalcified by 10% formic acid for 15 days and embedded in paraffin. The blocks were cut into thin sections and were stained by haematoxylin and eosin and histopathology was studied.

Statistical analysis: Results are expressed as mean ± SD. The level of significance was set at $p<0.05$. Graph Pad Prism-5 software was used for the statistical analysis.

RESULTS

Results for Rheumatoid arthritis:

Effect of pineapple juice, Diclofenac and their combination on body weight (in grams) of rats in Freund's adjuvant induced arthritis model. Mean weight of all the groups is comparable to each other at day 0, 1, 5, 12 and 21[table2]. There is no significant change in weight in any of the groups from day 0 to day 21.

Table 2: Effect of pineapple juice, diclofenac and their combination on body weight (in grams) of rats in Freund's adjuvant induced rheumatoid arthritis model.

Days	Group I Vehicle control Wt. in g	Group II Pineapple juice Wt. in g	Group III Diclofenac Wt. in g	Group IV Pineapplejuice+ Diclofenac Wt. in g
Day 0	305.8 ± 91.34	279.3 ± 77.86	335.2 ± 109.9	353 ± 105.7
Day 1	308 ± 89.94	295.2 ± 82.04	336.2 ± 111.8	376.5 ± 102.1
Day 5	313 ± 89.17	330 ± 91.85	354.8 ± 117.2	400 ± 103.6
Day 12	317.8 ± 89.76	366.5 ± 99.83	387.2 ± 121.2	429.3 ± 107
Day 21	323.8 ± 89.17	375.8 ± 99.02	417 ± 117.2	436 ± 101.9

P>0.05 for all groups compared to each other using one way ANOVA

Effect of pineapple juice, Diclofenac and their combination on inflamed left hind paw volume (in ml) of rats in Freund's adjuvant induced arthritis model

The volume of inflamed left hind paw was significantly lower in group IV as compared to other groups on days 5, 12 and 21 [Table 3]. This clearly indicates the highest efficacy of the combination group (group IV) in combating inflammation as against other study groups, followed by Diclofenac group (III) and lastly by pineapple group (II).

Table 3: Effect of pineapple juice, diclofenac and their combination on inflammed left hind paw volume (in ml) of rats in Freund's adjuvant induced rheumatoid arthritis model.

Days	Group I Vehicle control Vol in ml	Group II Pineapple juice Vol in ml	Group III Diclofenac Vol in ml	Group IV Pineapple juice + Diclofenac Vol in ml
Day 0	1.007 ± 0.32	0.74 ± 0.10	0.75 ± 0.08	0.79 ± 0.097
Day 1	1.07 ± 0.34	0.87 ± 0.17	0.81 ± 0.105	0.83 ± 0.11
Day 5	1.53 ± 0.33	1.003 ± 0.19*	0.88 ± 0.11*	0.87 ± 0.11*
Day 12	1.74 ± 0.36	1.08 ± 0.17*	1.03 ± 0.21*	0.925 ± 0.11*
Day 21	1.89 ± 0.36	1.14 ± 0.17*	1.05 ± 0.19*	0.97 ± 0.12*

* p<0.001 as compared to group 1 using one way ANOVA and post hoc Tukey's test.

Effect of pineapple juice, Diclofenac and their combination on right hind paw volume (in ml) of rats in Freund's adjuvant induced arthritis model. Mean right hind paw volume of all the groups was comparable to each other at day 0, 1, 5, 12 and 21[Table 4].

Effect of pineapple juice, Diclofenac and their combination on gait of rats in Freund's adjuvant induced arthritis model.

Gait scoring was done as following ; 0-Three legged gait, 0.5-Marked limping & 1-Normal gait (minimum score indicates more damage).

Score in Group 1 has substantially decreased from 1 to 0 from Day 0 to Day 21 indicating maximum damage, while in Group II, III and IV, scores are statistically higher [Table 5]. In Group IV score being highest (0.66±0.40) indicating minimum damage as against other groups. This is followed by Diclofenac group (0.5±0.31) and lastly by Pineapple group (0.08±0.20). This shows that the pineapple juice has an additive antiinflammatory effect with Diclofenac. By day 21st, lame gait was seen in all animals in the vehicle control group (group I). It was seen in 5 animals in the pineapple group (group II), 1 in Diclofenac group (group III) and 1 in the combination group (group IV).

Table 4: Effect of pineapple juice, diclofenac and their combination on right hind paw volume (in ml) of rats in Freund's adjuvant induced rheumatoid arthritis model

Days	Group I Vehicle control Vol in ml	Group II Pineapple juice Vol in ml	Group III Diclofenac Vol in ml	Group IV Pineapple juice + Diclofenac Vol in ml
Day 0	1.027 ± 0.27	0.86 ± 0.19	0.91 ± 0.25	1.01 ± 0.25
Day 1	1.05 ± 0.27	0.90 ± 0.215	0.91 ± 0.246	0.98 ± 0.22
Day 5	1.047 ± 0.25	0.87 ± 0.18	0.925 ± 0.26	0.92 ± 0.20
Day 12	1.027 ± 0.31	0.88 ± 0.20	0.94 ± 0.26	1 ± 0.22
Day 21	1.067 ± 0.28	0.91 ± 0.18	0.87 ± 0.23	1.002 ± 0.26

p>0.05 for all groups compared to each other using one way ANOVA.

Table 5: Effect of pineapple juice, diclofenac and their combination on gait of rats in Freund's adjuvant induced rheumatoid arthritis model

Days	Group I – Distilled Water	Group II – Pineapple Juice	Group III – Diclofenac Sodium	Group IV – Pineapple Juice + Diclofenac Sodium
Day 0	1 ± 0	1± 0	1± 0	1± 0
Day 1	1 ± 0 NS	1± 0 NS	1± 0 NS	1± 0 NS
Day 5	0.25 ±0.27‡	0.33 ± 0.25‡	0.41± 0.37 †	0.50± 0.44*
Day12	00‡	0.08 ± 0.20 ‡	0.5± 0.31†	0.66± 0.40 NS
Day 21	00‡	0.08 ± 0.20 ‡	0.5± 0.31†	0.66± 0.40 NS

Values are expressed as mean ±SD. For Statistical analysis one way ANOVA with post hoc Dunnett test was applied to compare gait scores on Day 0 with gait scores on Day-1/ Day-5/ Day-12/ Day- 21. *P < 0.05, † P < 0.01, ‡ P < 0.001. Ns: not significant Gait scoring; 0-Three legged gait, 0.5-Marked limping & 1-Normal gait (minimum score indicates more damage**)**

Results for Osteoarthritis:

As can be seen in Table 6, for most of the parameters, the drug intervention groups showed statistically significant favorable results as compared to the vehicle control group. For parameters like structural changes in the joints, disorganization of chondrocytes, degeneration of chondrocytes, necrosis, inflammatory cells infiltration in synovial tissue and safranin-O staining, Diclofenac group and the combination therapy group showed significant results as compared to pineapple group.

This shows that the Diclofenac group and the combination group showed better results on histology as compared to the pineapple group. However for parameters like structural changes in the joints, necrosis and synovial cell proliferation the combination therapy group (IV) showed statistically significant results as compared to Diclofenac group(III). It was interesting to know that the combination therapy group had a significantly lower histopathological score as compared to both Diclofenac and pineapple juice group. This shows that pineapple juice has an additive anti-inflammatory effect with Diclofenac.

Table 6: Histopathological score after administration of pineapple juice, Diclofenac and their combination on MIA-Induced osteoarthritis

No.	Parameters	Group I Vehicle control	Group II Pineapple juice	Group III Diclofenac	Group IV Pineapple juice + Diclofenac
1	Structural change in the joint (surface irregularities)	0.78 ± 0.07	0.53 ±0.08*	0.31 ± 0.102*†	0.135 ± 0.05*†‡
2	Ulceration	0.28 ± 0.04	0.10 ± 0.08*	0.035 ±0.054*	0.05 ± 0.054*
3	Fibrillation of cartilage surface	0.28 ± 0.058	0.16 ± 0.08	0.086 ± 0.10*	0.053 ± 0.09*
4	Disorganization of chondrocytes	0.728 ± 0.19	0.59 ± 0.07	0.19 ± 0.13*†	0.05 ± 0.05*†
5	Exposure of subchondral bones	0 ± 0	0 ± 0	0 ± 0	0 ± 0
6	Cellular changes of chondrocytes hypertrophy	0.49 ±0.20	0.20 ± 0.08	0.17 ± 0.07*	0.126 ±0.051*
7	Degeneration Necrosis	0.97 ± 0.03	0.74 ± 0.14*	0.22 ± 0.13*†	0.05 ±0.08*†‡
8	Inflammatory cell infiltration in synovial tissue	0.41 ± 0.06	0.36 ± 0.09	0.107 ± 0.089*#	0.083 ± 0.075*†
9	Synovial cell proliferation	0.66 ± 0.16	0.64 ± 0.13	0.39 ± 0.32	0.05± 0.05*†‡
10	Safranin-O staining (reduction of staining in cartilage)	0.93 ± 0.08	0.20 ± 0.12*	0.05 ± 0.05*†	0.05 ± 0.08*†
11	Total Pathology Score	5.71 ± 0.76	3.56 ± 0.44	1.56 ± 0.58	0.60 ± 0.29

* $p<0.05$ as compared to group 1 using One way ANOVA and post hoc Tukey's test, † $p<0.05$ as compared to group 2 using One way ANOVA and post hoc Tukey's test, ‡ $p<0.05$ as compared to group 3 using One way ANOVA and post hoc Tukey's test

DISCUSSION

The present study was planned to evaluate the anti-inflammatory effect of pineapple juice in inflammatory models of both rheumatoid and osteoarthritis. Even though bromelain, the extract of pineapple plant is used widely as a food supplement, it has a limitation of being costly as the extraction and purification process is expensive, it is about 2400 US dollars/kg [10]. Further, bromelain is used mainly as an additive to other medications [11]. Taking all these factors into consideration it was postulated that if only pineapple juice could show beneficial effects as additive to anti-inflammatory effects then one could use pineapple juice instead of bromelain extract, thereby reducing the total cost of treatment. In our study it was found that in the monoiodoacetate model of osteoarthritis the total histopathology score of the combination therapy group was significantly lower as compared to Diclofenac group, thus showing that the pineapple juice indeed has an additive effect. However, similar effects were not seen in the rheumatoid arthritis model even though there was a trend showing a decrease in the paw volume.

Animals were observed for paw edema, gait & weight gain after injecting them with Complete Freund's adjuvant. The acute inflammatory response gets initiated immediately within a day resulting in the paw becoming red and edematous [12]. This extends to involve the synovium and intra-articular tissues by day 5.

This inflammation further extends to the para-articular tissues and bones in subchondral region. The damage becomes extensive by day 12. There is involvement of other non-injected joints as well [13]. This entire manifestation resembles rheumatoid arthritis [14] .

Comparing the rat hind paw volume, in our results showed that effects of the pineapple juice, Diclofenac and combination therapy started to appear from Day 5 through day 21. Even though the treatment was continued only till day 12, the effects were present till day 21. All the three groups were equi-efficacious. The anti-inflammatory effects of pineapple juice can be attributed to decreased expression of PGE2 due to the bromelain component of pineapple juice[15]. PGE2 has been implicated in the pathogenesis of rheumatoid arthritis particularly in disease progression and antagonists of the PGE2 receptor i.e. EP4 receptors are being evaluated for treatment of rheumatoid arthritis[16].

The gait of all the animals changed after injecting the left hind paw with complete Freund's adjuvant. By the 21st day, lame gait was seen in all animals in the vehicle control group (group I). It was seen in 5 animals in the pineapple juice group (group II), 1 in Diclofenac group (group III) and 1 in the combination group (group IV). This shows that that the anti-inflammatory effects were similar due to both Diclofenac and combination therapy group, again reinforcing the concept that pineapple juice can be used as an add on anti-inflammatory adjuvant to Diclofenac.

The animal model of monoiodoacetate-induced arthritis resembles osteoarthritis closely with it showing both the histological changes and symptoms of osteoarthritis. This model hence can be used for evaluation of mechanisms

involved in osteoarthritis and testing of drugs that can be used in the treatment of osteoarthritis [17].

Presently, there is no treatment available which can stop the progress of osteoarthritis. Most of the treatments are directed towards pain relief with improvement in joint mobility [18]. The present study evaluated the effects of pineapple juice alone and in combination with Diclofenac in the treatment of osteoarthritis.

In this study pineapple juice has been shown to decrease several histological inflammatory parameters. Also the effects of pineapple juice and Diclofenac combination therapy groups were much more significant as compared to Diclofenac group. Mitogen activated protein kinase (MAP kinase) has been implicated in the pathogenesis of osteoarthritis [19]. There is evidence that pineapple juice extract bromelain decreases MAP kinase pathway activity, which might be the reason for its additive effect to Diclofenac [20].

There are several other studies that have shown the effects on pineapple extract bromelain in osteoarthritis. A randomized clinical trial has shown that bromelain containing preparations are equally effective as compared to Diclofenac in knee osteoarthritis patients [21]. Tilwe et al have also shown that daily bromelain administration showed significantly better improvement in osteoarthritic pain, swelling and tenderness as compared to Diclofenac [22]. These results corroborate with results of our findings.

CONCLUSION

The present study shows that the pineapple juice has anti-inflammatory properties. It shows comparable or even better results than Diclofenac if used in combination with Diclofenac in models of osteoarthritis and rheumatoid arthritis.

CONFLICT OF INTEREST: There was no conflict of interest among authors.

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