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Anti-Arthritis, Antioxidant and Anti - Inflammatory Potential of Ethanolic Extract of Guava Leaves on Rats Exposed to High Fat Diet and Freud Adjuvant

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Guava leaves (*Psidium guajava*) have been used traditionally for years to treat common ailments such as *Diabetes*, diarrhoea, and hypertension. This study was designed to determine the anti-arthritic, anti-inflammatory, and antioxidant effect of the ethanolic extract of guava leaf (*Psidium guajava*) on rats fed with a high-fat diet and induced arthritis using complete Freud Adjuvant. Seventy-two male and female albino rats were used in this study, the rats were grouped into 12 with 6 rats in each group, rats were fed a high-fat diet to cause hyperlipidaemia and induced rheumatoid arthritis by injecting 0.1ml of Complete Freund's Adjuvant into their right hind paw.

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Group 1 was fed with normal feed and water, group 2 was made of healthy rats, fed with normal feed, water, and guava leaf extract, group 3 was fed with a high-fat diet and induced arthritis with complete Freund's Adjuvant (CFA) and water, group 4 received dexamethasone (6.75 mg.kg-1 orally), and groups 5 to 12 received the extract at oral doses of 250 and 750 mg/kg, respectively for a period of 28days. ELISA technique was used to analyse the inflammatory markers, antioxidants; SOD, and MDA, while the lipid profile was on a spectrophotometer. The inflammatory markers TNF-α, IL-6, and C-reactive protein were significantly reduced in test subjects at $p<0.05$, HDL and SOD had no statically significant difference, while MDA was markedly reduced at $p<0.05$. This study demonstrates that *Psidium guajava* extract has significant anti-arthritic and lipid-lowering effects. *Psidium guajava* leaves can be developed into an alternative anti-arthritis and lipid-lowering treatment.

Keywords: Antioxidant; arthritis; anti-arthritis; Freud adjuvant; guava; high-fat diet; Inflammatory.

1. INTRODUCTION

Arthritis is an inflammatory disorder affecting one or more joints of the body with varying causal factors, including trauma, infections, autoimmune disorders, idiopathic causes, and aging. Irrespective of the cause, the underlying pathophysiology involves the breakdown of cartilage, which protects the end surfaces of bones at the joints, leading to the loss of smooth glide at the joint during movement. This frictional rubbing results in pain, swelling, and stiffness at the joint and eventual muscle strain due to difficulty moving the joint [1]. Two of the most common types are osteoarthritis and rheumatoid arthritis.

Rheumatoid Arthritis (RA) is most commonly seen in adults over the age of 65, but it can also develop in children, teens, and younger adults [2]. Alternative methods to address RA, such as the consumption of medicinal plants are becoming popular [3].

Plant extracts have been used as a source of medicines for a wide variety of human ailments. Among the numerous traditional medicinal herbs*, is Psidium guajava*, commonly known as Guava, which is used for the treatment of numerous diseases in Africa, Asia, and other countries [4].

However, neither preventive measures nor primary cures for RA have been established. Hence, alternative methods to address RA, such as the use of medicinal plants [5].

Psidium guajava (common name guava) is a well-known tropical tree that is abundantly grown for fruit. It belongs to the phylum Magnoliophyta, class Magnoliopsida, and Myrtaceae family [6]. It has about 133 genera and more than 3,800 species. Guava contains a large number of antioxidants and phytochemicals including essential oils, polysaccharides, minerals, vitamins, enzymes, triterpenoid acid alkaloids, steroids, glycosides, tannins, flavonoids, and saponins. Guava contains a higher content of vitamins C and A. Guava is also a very good source of pectin, and important dietary fibre. It has a high content of flavonoids, fructose, and carotenoids. The guava fruit contains vitamins A, and C, iron, phosphorus, and calcium. It has more vitamin C than the orange [7].

Rheumatoid arthritis (RA), the most common type of joint disease, is on the increase across different age ranges. RA affected about 24.5 million people as of 2015. This is between 0.5 and 1% of adults in the developed world with 5 and 50 per 100,000 people newly developing the condition each year. Onset is most frequent during middle age and women are affected 2.5 times as frequently as men. Proper management and treatment are expensive with conventional drugs having lots of side effects. Thus the quest for a cheaper and safer alternative. This provoked our interest in investigating the antioxidant, anti-inflammatory and anti-arthritis potential of guava leaf.

2. THE EXPERIMENTAL ANIMALS

The study was conducted on seventy- two albino Wistar rats weighing 130-210 g purchased from the animal house in the Department of Physiology University of Port Harcourt Rivers state. Animals were acclimatized to experimental conditions in cages and kept under standard environmental conditions (25 ± 3ºC; 12/12 h light/dark cycle). Rats were allowed to feed and water ad libitum. The rats were grouped into 12 groups of 6 rats each.

Group 1; Negative control (healthy rats fed with normal feed and water)

Group 2; Normal control (healthy rats fed with normal feed and water treated with ethanolic extract of guava leaf 250mg/kg)

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Group 3; Positive control (Rats fed with high fat diet and induced arthritis with complete Freund's Adjuvant (CFA) and water)

Group 4; Rats fed with high fat diet and induced arthritis with complete Freund's Adjuvant (CFA) treated with dexamethasone (6.75mg/kg body weight).

Group 5; low dose (250mg/kg body weight) for 1 week on rats on HFD and induced arthritis

Group 6; high dose for (750mg/kg body weight) 1 week on rats on HFD and induced arthritis

Group 7; low dose (250mg/kg body weight) for 2 weeks on rats on HFD and induced arthritis

Group 8; high dose (750mg/kg body weight) for 2 weeks on rats on HFD and induced arthritis

Group 9; low dose for 3 weeks on rats on HFD and induced arthritis

Group 10; high for dose 3 weeks on rats on HFD and induced arthritis

Group 11; low dose for 4 weeks on rats on HFD and induced arthritis

Group 12; high dose for 4 weeks on rats on HFD and induced arthritis

Each group was administered 0.2ml CFA(1mg/ml)

2.1 Evaluation of Arthritic Score

Each paw was scored on a scale of 0–4 based on their degree of swelling, erythema, and deformity (maximum score 16 per animal) as follows: 0 = normal, 1 = slight erythema/ swelling of the ankle, 2 = moderate erythema and/or swelling of ankle, 3 = severe erythema/ swelling of ankle and 4 = complete erythema and swelling of the toes and inability to bend the ankle. The arthritic score was measured on days 0, 1, 4, 8, 12, 16, 20, 24, and 28.

Evaluation of mobility score; Whole animal mobility was scored between 0 and 4 according to the following definitions: 0 normal, 1 = slightly impaired, 2 = major impairment, 3 = does not step on paw, and 4 =no movement. The mobility score was measured on days 12 and 28.

2.2 Preparation of Guava Leaves Extract

Fresh tender leaves of guava were collected from Elelenwo, Port Harcourt Rivers State Nigeria,1 kg. The plant was authenticated by Botanists and a herbarium number RSUPB0103 was obtained.

The plant was washed with distilled water and air-dried at room temperature for 14 days. Extracts were prepared following the method described by Salem et al. [8] 1000g of dried ground guava leaves were macerated using 96% ethanol solvent for 48 h and then filtered with a Buchner funnel to obtain the filtrate. The filtrate obtained was then concentrated using a rotary evaporator at a maximum temperature of 60°C until the ethanol solvent evaporated and a paste-shaped extract was formed, the paste extract was then taken using a spatula then weighed and refrigerated until time it was used.

2.3 High Fat Diet Using Egg Yolk

The normal rat chaw was measured and 20 percent of the feed was removed and replaced with egg yolk to induce hypercholesteremia.

3. RESULTS

3.1 Antioxidant and Anti-Inflammatory Variables of Rats Treated with Low Dose Ethanol Leaf Extract of *Psidium guajava*

The mean and standard deviation of MDA, SOD, CRP, IL6, and TNF-α of groups 5, 7, 9, and 11 rats were compared with the control groups 1, 2, and 3 and are shown in detail in Table 4. There were significant variations among the mean values of MDA (Malondialdehyde) at P-value = 0.0001. Similarly, there were statistically significant differences amongst the mean values of CRP (C reactive protein) and TNF-α (Tumor Necrosis Factor) at a p-value of 0.0001 respectively. However, in the control group 1, 2, and 3 when compared with the test groups 5,7,9,11 there was no statistically significant difference in the mean values of SOD (Superoxide Dismutase) and Interleukin-6 (IL6) at p – values at 0.1852 and 0.3376 respectively. Details of the ANOVA result of antioxidant and anti-inflammatory variables of rats treated with low-dose ethanol leaf extract of *Psidium guajava* are shown in Table 1.

Table 1. Antioxidant and anti-inflammatory variables of rats treated with low-dose ethanol leaf extract of *Psidium guajava*

	MDA(nmol/ml)	SOD (ng/L)	IL6 (ng/ml)	CRP(Ug/L)	TNF-α (ng/L)
Group 1	0.67 ± 0.02b	8.45 ± 0.81b	15.24 ± 1.26b	0.31 ± 0.01	22.43 ± 0.79b
Group 2	0.95 ± 0.1a	9.0 ± 1.41b	23.16 ± 0.43a	0.76 ± 0.5a	24.43 ± 1.66b
Group 3	0.53 ± 0.03a	6.55 ± 1.07b	19.14 ± 0.49b	0.33 ± 0.03b	33.105 ± 2.34b
Group 5	0.47 ± 0.05a	6.22 ± 0.91b	19.02 ± 1.42b	0.56 ± 0.3a	22.37 ± 1.8b
Group 7	0.61 ± 0.1a	6.01 ± 0.61b	21.03 ± 2.55b	0.63 ± 0.13a	17.06 ± 0.78b
Group 9	0.66 ± 0.05a	8.25 ± 1.51b	18.49 ± 0.64	0.41 ± 0.07b	24.57 ± 0.59b
Group 11	0.77 ± 0.06a	6.21 ± 0.32b	17.45 ± 0.55b	0.53 ± 0.07a	20.96 ± 2.39b
p-values	0.0001	0.1852	0.3376	0.0001	0.0001
F-values	6.844	1.583	1.19	8.045	7.348

Key: Group1 – Negative control, Group 2 – Normal control fed with extract, Group 3 – Positive control Group 5 – induced rats fed with a low dose of extract for 1 week, Group 7 – induced rats fed with a low dose of extract for 2 weeks, Group 9 – induced rats fed with a low dose of extract for 3 weeks, Group 11 – induced rats fed with a low dose of extract for 4 weeks, a= statistically significant , b– not significant

3.2 ANOVA Results of Antioxidant and Anti-Inflammatory Variables of Rats Treated with High Dose Ethanol Leaf Extract of *Psidium guajava*

The mean and standard deviation of MDA, SOD, CRP, IL6, and TNF-α of groups 6, 8,10, and 12 rats were compared with the mean and standard deviation of the control groups 1, 2, and 3. There were significant variations among the mean values of MDA (Malondialdehyde) at p- value = 0.0001. Similarly, there were statistically significant differences amongst the mean values of IL6 at a p-value of 0.0155, CRP (C reactive protein), and TNF-α (Tumor Necrosis Factor) at a P-value of 0.0001 respectively. However, in the control group 1, 2, and 3 when compared with the test groups 6, 8,10, and 12 there was no statistically significant difference in the mean values of SOD (Superoxide Dismutase) at p – values at 0.1852. Details of the ANOVA result of antioxidant and anti-inflammatory variables of rats treated with high-dose ethanol leaf extract of *Psidium guajava* are shown in Table 2.

3.3 Antioxidant and Anti-Inflammatory Variables of Groups 5 and 6 Rats Treated with Ethanolic Leaf Extract of *Psidium guajava* week1

Comparative analysis of the mean and standard deviation of MDA, SOD, Interleukin 6, C reactive protein, and TNF-α of groups 1,2,3,5, and 6. There was a statistically significant variation in the mean values of MDA (p – value at 0.0001) and mean values of TNF-α (p – value at 0.0008). However, there was no significant difference in the mean values of SOD (p – value at 0.271) and mean values of IL6 (p – values at 0.1123) Details of the comparative analysis are in Table 3.

Table 2. Antioxidant and anti-inflammatory variables of rats treated with high dose ethanolic leaf extract of *Psidium guajava*

	MDA(nmol/ml)	SOD (ng/L)	IL6 (ng/ml)	CRP (Ug/L)	TNF-α (ng/L)
Group 1	0.67 ± 0.02 [a]	8.45 ± 0.81[b]	15.24 ± 1.26 [b]	0.31 ± 0.01 [a]	22.43 ± 0.79 [b]
Group 2	0.95 ± 0.1[a]	9.0 ± 1.41 [b]	23.16 ± 0.43 [b]	0.76 ± 0.5 [a]	24.43 ± 1.66 [b]
Group 3	0.53 ± 0.03 [a]	6.55 ± 1.07 [b]	19.14 ± 0.49 [b]	0.33 ± 0.03 [b]	33.105 ± 2.34 [a]
Group 6	0.57 ± 0.06 [a]	9.87 ± 2.11 [b]	22.04 ± 4.3 [b]	0.62 ± 0.03 [a]	22.83 ± 1.85 [a]
Group 8	0.65 ± 0.02 [a]	8.34 ± 0.75 [b]	20.39 ± 1.47 [b]	0.64 ± 0.1 [a]	19.71 ± 1.54 [a]
Group 10	0.58 ± 0.13 [b]	8.59 ± 0.5 [b]	14.31 ± 1.11 [b]	0.58 ± 0.11 [a]	29.42 ± 5.46 [b]
Group 12	0.59 ± 0.04 [a]	5.89 ± 0.69 [b]	15.50 ± 1.26 [b]	0.47 ± 0.03 [b]	19.02 ± 1.6 [a]
p-values	0.0004	0.2378	0.0155	0.0001	0.0002
F-values	5.69	1.414	3.155	8.178	6.138

Key: Group1 – Negative control, Group 2 – Normal control fed with extract, Group 3 – Positive control Group 6 – induced rats fed with a high dose of extract for 1 week, Group 8 – induced rats fed with a high dose of extract for 2 weeks, Group 10 – induced rats fed with a high dose of extract for 3 weeks Group 12 – induced rats fed with a high dose of extract for 4 weeks, a = statistically significant, b – Not significant

Table 3. Antioxidant and anti-inflammatory variables of Groups 5 and 6 rats treated with ethanol leaf extract of *Psidium guajava*

	MDA(nmol/ml)	SOD (ng/L)	IL6 (ng/ml)	CRP(Ug/L)	TNF-α (ng/L)
Group 1	0.67 ± 0.02b	8.45 ± 0.81 [b]	15.24 1.26 [b]	0.31 ± 0.01 [b]	22.43 ± 0.79 [b]
Group 2	0.95 ± 0.1a	9.0 ± 1.41 [b]	23.16 ± 0.43 [b]	0.76 ± 0.5a	24.43 ± 1.66 [b]
Group 3	0.53 ± 0.03a	6.55 ± 1.07 [b]	19.14 ± 0.49 [b]	0.33 ± 0.03 [b]	33.105± 2.34a
Group 5	0.47 ± 0.05a	6.22 ± 0.91 [b]	19.02 ± 1.42 [b]	0.56 ± 0.3a	22.37 ± 1.8 [b]
Group 6	0.57 ± 0.06	9.87 ±2.11 [b]	22.04 ± 4.3 [b]	0.62± 0.03a	22.83 ± 1.85 [b]
p-values	0.0001	0.271	0.1123	0.0001	0.0008
F-values	10.19	1.317	2.047	36.48	6.778

*Key: Group1 – Negative control, Group 2 – Normal control fed with extract, Group 3 – Positive control Group 6 – induced rats fed with a high - dose of extract for 1 week, Group 5 – induced rats fed with a low - dose of extract for 1 week, *= statistically significant, ns – Not significant*

3.4 Mean of Antioxidant and Anti-Inflammatory Variables of Group 7 and 8 Rats Treated with Ethanolic Leaf Extract of *Psidium guajava*

Comparative analysis of the mean and standard deviation of MDA, SOD, Interleukin 6, C reactive protein, and TNF-α of groups 1,2,3,7, and 8. There was a statistically significant decrease in the mean values of MDA (p –value at 0.0001), CRP (p – value at 0.0001), mean values of TNF-α (p – value at 0.0008), and mean values of IL6 (p –values at 0.0023) in the test group 7,8 when compared with control group 1,2,3. However, there was no significant difference in the mean values of SOD (p – value at 0.2338) Details of the comparative analysis are in Table 4.

3.5 Antioxidant and Anti-Inflammatory Variables of Groups 9 and 10 Rats Treated with Ethanol Leaf Extract of *Psidium guajava*

Comparative analysis of the mean and standard deviation of MDA, SOD, Interleukin 6, C reactive protein, and TNF-α of groups 1,2,3,9, and 10. There was a statistically significant decrease in the mean values of MDA (p –value at 0.002), CRP (p – value at 0.0001), mean values of TNF-1(P – value at 0.0001), and mean values of IL6 (p –values at 0.0023) in the test groups 9 and 10 when compared with control group 1,2,3. However, there was no significant difference in the mean values of SOD (P – value at 0.5123) Details of the comparative analysis are in Table 5.

3.6 Antioxidant and Anti-Inflammatory Variables of Rats in Groups 11 and 12 Treated with Ethanolic Leaf Extract of *Psidium guajava*

Comparative analysis of the mean and standard deviation of MDA, SOD, Interleukin 6, C reactive protein, and TNF-α of groups 1, 2, 3, 11, and 12. There was a statistically significant decrease in the mean values of MDA (p –value at 0.002), CRP (p – value at 0.0001), mean values of TNF-1(p – value at 0.00002), and mean values of IL6 (p –values at 0.0001) in the test group 11 and 12

Table 4. Antioxidant and anti-inflammatory variables of Groups 7 and 8 rats treated with ethanolic leaf extract of *Psidium guajava*

	MDA (nmol/ml)	SOD (ng/L)	IL6 (ng/ml)	CRP (Ug/L)	TNF-α (ng/L)
Group 1	0.67±0.02[b]	8.45±0.81[b]	15.24 ± 1.26[b]	0.31 ± 0.0[b]	22.43 ± 0.79[b]
Group 2	0.95 ± 0.1[a]	9.0 ± 1.41[b]	23.16 ± 0.43[a]	0.76 ± 0.5[a]	24.43 ± 1.66[b]
Group 3	0.53 ± 0.03[b]	6.55±1.07[b]	19.14 ± 0.49[b]	0.33 ± 0.03[b]	33.105 ± 2.34[a]
Group 7	0.61 ± 0.1[b]	6.01±0.61[b]	21.03 ± 2.55[b]	0.63 ± 0.13[a]	17.06 ± 0.78[a]
Group 8	0.65 ± 0.02[b]	8.34±0.75[b]	20.39 ± 1.47[b]	0.64 ± 0.1[a]	19.71 ± 1.54a
p-values	0.0008	0.2338	0.0023	0.0002	0.0001
F-values	6.947	1.504	5.743	5.837	13.61

*Key: Group1 – Negative control, Group 2 – Normal control fed with extract, Group 3 – Positive control Group 7 – induced rats fed with a low- dose of extract for 2 weeks, Group 8 – induced rats fed with a high – dose of extract for 2 weeks, *= statistically significant, ns – Not significant*

Table 5. Antioxidant and anti-inflammatory variables of Groups 9 and 10 rats treated with ethanolic leaf extract of *Psidium guajava*

	MDA(nmol/ml)	SOD(ng/L)	IL6 (ng/ml)	CRP(Ug/L)	TNF-α (ng/L)
Group 1	0.67 ± 0.02^{b}	8.45 ± 0.8^{b}	15.24 ± 1.26^{b}	0.31 ± 0.01^{b}	22.43 ± 0.79^{b}
Group 2	0.95 ± 0.1^{a}	9.0 ± 1.41^{b}	23.16 ± 0.43^{a}	0.76 ± 0.5^{a}	24.43 ± 1.66^{b}
Group 3	0.53 ± 0.03^{b}	6.55 ± 1.07^{b}	19.14 ± 0.49^{a}	0.33 ± 0.03^{b}	33.105 ± 2.34^{a}
Group 9	0.66 ± 0.05^{b}	8.25 ± 1.51^{b}	18.49 ± 0.64^{a}	0.41 ± 0.07^{b}	24.57 ± 0.59^{b}
Group 10	0.58 ± 0.13^{b}	8.59 ± 0.5^{b}	14.31 ± 1.11^{b}	0.58 ± 0.11^{a}	29.42 ± 5.46^{b}
p-values	0.002	0.5123	0.0001	0.0001	0.0142
F-values	6.017	0.8183	18.12	11.18	3.971

Key: Group1 – Negative control, Group 2 – Normal control fed with extract, Group 3 – Positive control Group 9 – induced rats fed with low dose of extract for 3 weeks, Group 10 – induced rats fed with high dose of extract for 3 weeks, a = statistically significant, b – Not significant

Table 6. Antioxidant and anti-inflammatory variables of rats in groups 11 and 12 treated with ethanolic leaf extract of *Psidium guajava*

	MDA (nmol/ml)	SOD (ng/L)	IL6 (ng/ml)	CRP (Ug/L)	TNF-α (ng/L)
Group 1	0.67 ± 0.02b	8.45 ± 0.81b	15.24 ± 1.26b	0.31 ± 0.01b	22.43 ± 0.79b
Group 2	0.95 ± 0.1a	9.0 ± 1.41b	23.16 ± 0.43a	0.76 ± 0.5a	24.43 ± 1.66b
Group 3	0.53 ± 0.03b	6.55 ± 1.07b	19.14 ± 0.49b	0.33 ± 0.03b	33.105 ± 2.34a
Group 11	0.77 ± 0.06a	6.21 ± 0.32b	17.45 ± 0.55b	0.53 ± 0.07a	20.96 ± 2.39b
Group 12	0.59 ± 0.04b	5.89 ± 0.69a	15.50 ± 1.26b	0.47 ± 0.03b	19.02 ± 1.6b
p-values	0.0002	0.0896	0.0001	0.0001	0.00002
F-values	8.066	2.275	13.47	16	8.66

*Key: Group1 – Negative control, Group 2 – Normal control fed with extract, Group 3 – Positive control Group 11 – induced rats fed with a low dose of extract for 4 weeks, Group 12 – induced rats fed with a high dose of extract for 4 weeks, *= statistically significant, ns – Not significant*

when compared with control group 1,2,3. However, there was no significant difference in the mean values of SOD (p– value at 0.0896) Details of the comparative analysis are in Table 6.

4. DISCUSSION

The use of medicinal plants continues to spread globally as more research has shown that their effectiveness in most disease conditions is not folktale. Inflammatory and arthritic conditions are among ailments treated using traditional remedies, with considerable success. Chronic inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. Due to the changes in lifestyle and social and economic conditions, nowadays obesity is a global epidemic problem affecting both developing and developed nations. High-fat diet-induced obesity in animals and complete Freud adjuvant-induced arthritis have been considered as the method of choice among researchers because they produce high similarity of mimicking the usual route of obesity and arthritis in humans [9]. In this study administration of guava leaf extract can be seen to significantly reduce the effect of anti-inflammatory, antioxidant, and arthritis.

In Table 1 comparison was made between the control groups and test groups that were administered low doses of guava leaf extract at different weeks; a comparison of MDA between the control group and test group showed a significant decrease ($p<0.05$) this agrees with the work of [10,11,12]and the result of decomposing end peroxide formed during the process of lipid peroxidation produces MDA therefore, it is very appropriate to be used as an indicator of lipid peroxidation [13].

Comparison of SOD in Table 1 shows no significant difference ($p>0.05$) even though the marked increase in SOD was noted in group 2 these results disagree with the report by [15] who had a statistically significant mean increase, this could be due to specie of guava leaf used but the report of [14] agrees, their report says no significant difference. The anti-inflammatory markers CRP, TNF-α, and IL6 had a statistically significant decrease ($p<0.05$) mean of test groups at different weeks when compared to control groups this agrees with the study by [15].

The anti-inflammatory action of flavonoids found in guava leaf extract is mainly due to its ability to inhibit the formation of proinflammatory mediators (e.g., adhesion molecules, cytokines, eicosanoids, and C-reactive protein). Phytochemical analysis of guava leaf extract shows a high content of flavonoids alongside alternative phytoconstituents, which can be responsible for its, antioxidative and anti-inflammatory properties [16].

In Table 3 comparative analysis was done on the control groups and the test group administered a high dose of guava leaf extract; There was no significant difference in the mean SOD when compared. Superoxide dismutases (SODs) are a group of metalloenzymes that are found in living cells. They form the front line of defense against reactive oxygen species (ROS)-mediated injury. These proteins catalyze the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2) and decrease the O_2^- level which damages the cells at excessive concentrations [17]. However, the report from this study by Olaniyan [18], suggests that the administration of Psidium guajava extract increases SOD activity. However, there was a significant decrease in CRP, MDA, IL6, and TNF-α and this is similar to the study done by Naseer et al. [19].

Tables 5, and 6, of this study showed no statistically significant difference ($p>0.05$) in the SOD mean ± SD in the test and control groups when a comparative analysis was done based on the different weeks at a dosage of 750mg. This study is in agreement with [18,20]; there was statistically significant variation in the CRP, TNF-α, and IL6 this study by [21] supports this. Several studies have shown the antioxidant and anti-inflammatory properties of flavonoids found in *P. guajava* leaf extract as well as triterpenoids, vitamin C, and tannins, contribute to its anti-inflammatory effect, although the actual mechanism of suppression of the arthritic condition is not known.

5. CONCLUSION

Results from this study show that *Psidium guajava* administration can improve the antioxidant profile and decrease inflammation. The extract also contains many secondary metabolites, such as flavonoids, triterpenoids, sesquiterpenes, glycosides, alkaloids, saponins, and other phenolic compounds. These compounds have been found to play key roles in the amelioration of several disease conditions. Unlike drugs that may induce adverse side effects, the safety of guava leaf extract has been proven by its application in folk medicine and the diet of various regions. However, future studies involving human subjects are needed to confirm the effectiveness of guava leaf extract in the treatment of RA. These results support the hypothesis that *Psidium guajava* has a potential role in the treatment and management of arthritis and antioxidants in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethics Committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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