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The isolation characterization and assessment of bioactive flavonoid with special reference to anti-arthritic activity

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ABSTRACT

A particular attention should be paid to isolation, characterization, and assessment of bioactive flavonoids, especially for anti-arthritic activity. The slurry of adsorbent (silica gel; 60–120 lattice) was blended in n-hexane to form a uniform slurry, and this was then employed as a fixed-stage adsorbent. Butanolic solution (10 g) was dissolved in the minimum volume of butanol, and then adsorbed on silica gel (60–120 mesh) while still wet. The wet powder was then dried, and the separated liquid phase was collected and applied to the column to try to isolate possible phytoconstituents. A whole plate full of fractions was monitored simultaneously on a TLC plate with a mixture of chloroform:methanol:water (68:30:02) as the solvent system. Six fractions (F1–F6) were generated after the colours of F1 and F2 were mixed next to each other, and after R_f was discovered in F3. A separate group of six animals was created for the survey, which was made up of five treatment groups and one control group. It was concluded that yellow crystalline solid luteolin was obtained as a result, with the Shinoda test confirming that it was a flavonoid. The absorption of luteolin was discovered to be in an aqueous alkaline solution. There were five different resonances, occurring at different frequencies, in the 400 MHz ¹H NMR spectrum: 7.00 (d, J = 13.5 Hz, 2H), 6.73 (s, 1H), 6.56 (s, 1H), 6.18 (s, 1H), 6.05 (s, 1H), 4.78 (s, 1H), 4.24 (s, 1H), 3.81 (s, 1H) (s, 1H). Inhibiting arthritic inflammation at doses of 40 mg/kg (luteolin) was significant (P < 0.001), while the adjuvant arthritic regulation was no longer significantly different from the control on the 28th day.

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1. Introduction

Asian countries have a prosperous arrangement of therapeutic vegetation dispensed in special environmental and geological requirements monster in the country. Plants have been utilized broadly in light of the fact that ancient cases for a fix of the scope of ailments [4].

For quite a while homegrown products and common therapies utilized in individuals medicine have been utilized as the stock of numerous therapeutically fine medications. Glancing lower back ahead of time the leftover 2000 years of medication, humankind has especially utilized vegetation as the lovely inventory of medication. More than 248,000 types of more noteworthy blossoms have been perceived and from these, 12,000 are perceived to have

restorative properties. More than 2,000 plants found in Asian countries, and Tibbi primary health care is natural plant names in Indian, ancient Ayurvedic, Unani, and Tibbi medicinal systems.

In Asian countries, the earliest mentioned use of medicinal plants is originated in Rigveda which was written between 4500 and 1600BCE. A detailed explanation of the world's first symposium on medicinal plants is given in the first chapter of Vrihat Samhita and since 1600BCE the amount of literature on this subject is unlimited. Although seventy-five percent of the world's population relies on this native Asian countries system for treatment of a variety of illnesses, the traditional medical system remains so ingrained in their culture and tradition that it influences even people who have left the Asian countries [15]. With such a vast section of an ever-increasing population dependent on herbal remedies; it is essential that the plant products which have been in use for such a long time be scientifically proved for their efficacy [1].

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Medicinal plants have a bright future because there are about half a million plants in the world, and no researchers have analyzed their clinical utility. Further, as of this particular moment, their clinical applications have still not been investigated. If their clinical utility is found to be conclusive in future investigations, this could represent a paradigm shift in medicine [3]. The focus of the present study is to segregate the bioactive compound from the powerful extract, as well as to evaluate for the anti-arthritis activity [16–20].

2. Material & methods

2.1. Preparation of total crude extract

All plant materials (leaves, for example) were allowed to dry under shade, and they were then ground using a coarse powder for an extraction method. The methanol was placed in a Soxhlet apparatus and leaves of *Centella Asiatica* were extracted for 72 h using 95% methanol. Crude extracts were produced by reducing the pressure under which the methanolic extracts were dried. After drying, the corresponding extracts were weighed, and their respective percentage yield was ascertained [9].

2.2. Bioactivity guided isolation of phytoconstituents from *Centella asiatica*

2.2.1. Formulation of the column

The adsorbent was added to the n-hexane, blended, and set up as a fixed phase. After it was added to the glass column (90 cm × 3 cm), the substance was allowed to settle. Glass rod was used to stir the enclosed air, which resulted in the release of the trapped air. The technique of column filling known as the wet filling is referred to as the wet method [21,22]. A tiny quantity of sand was managed to keep at the top of the column, just in case, it was needed. Overstep was allowed to flow until the level of the liquid phase was above the sand layer at the top of the column and had collected above it [10].

2.3. Formulation of sample and loading

To differentiate probable phytoconstituents, a butanoic fraction (10 g) was disintegrated in a specific amount of butanol and adsorbed on silica gel (60–120 mesh), then allowed to dry.

2.4. Selection of mobile phase for separation of phytoconstituents

A concentrated solution of the active methanolic extract (20 g) of *Centella Asiatica* was applied to a glass column and made subject to chromatography to separate the components. Chloroform:methanol was used as a solvent in the scheme. The gradient elution was accompanied in order to isolate the population. Column elution of chloroform was originally carried out with pure solvent, and as the amount of methanol was increased (from 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40 to 50:50), the chloroform ran off. Of the total of 160 fractions of 20 ml each, 120 were collected. A whole plate full of fractions was monitored instantaneously on a TLC plate with a mixture of chloroform:methanol:water (68:30:02) as the reducing agents. The colors and R_f visualizations were found to be the same in all the fractions; the resulting six fractions (F1–F6) were pooled together.

The results were as follows: The preliminary phytochemical analysis showed that all the fractions (F1–F6) were comprised of identifiable plant compounds. Biochemical examination on fractions (F1–F6) was done to measure their protein denaturation activity.

2.5. Assessment of anti-arthritis activity of bioactive flavonoid

2.5.1. Selection of animals

We obtained Wistar rats 2 to 3 months old that weighed approximately 150 to 200 g from the focal creature house. All species were kept in a climate-controlled facility at a temperature of 25 to 10°C, with 12-hour light and 12-hour dark cycles. The critees were permitted to do business. They were given a rodent pellet diet and were not required to supply water. The enclosure materials were changed daily. All of the testings were done in accordance with CPCSEA regulations with the goal of overseeing and controlling animal testing.

2.6. Acute toxicity studies of Luteolin

The LD₅₀ of Luteolin was reported by previous researchers as 400 mg/kg respectively. So from the literature review, we selected the predefined dose of both compounds for anti-arthritis activity as 20 & 40 mg/kg (Luteolin) respectively.

2.7. FCA induced arthritis in rat

A total of six animals were assigned to each group, resulting in a total of 30 animals in the research. FCA (0.1 ml) was implanted intra-articularly into the left ankle joint of rats on the 0th day to induce a chronic inflammatory reaction. Prior to Freund's Comprehensive Adjuvant (FCA) injection, a pre-induction baseline (left paw volume of each animal) was quantified at 0 days for Wistar rats for the induction of arthritis. Was given once every day from the first day of inoculation until 28 days later. One percent Tween 80 was used to make suspensions of the tested compounds. Following are the various animal species [2]:

- Group I: Normal Regulation: Allowed to treat with salt water
- Group II: for the treatment of arthritic conditions, a total of 0.1 ml of FCA was applied on the 0th day.
- Group III: Basic control: prednisolone (10 mg/kg, p.o.) was used in combination with FCA
- Group IV: Luteolin (20 mg/kg, p.o.) was administered, followed by FCA
- Group V: Administered Luteolin (40 mg/kg, oral) along with FCA

2.8. Estimation of biophysical parameters

The procedure of measurement of paw volume (0th, 7th, 14th, 21th, 28th day) arthritis assessment (7th, 14th, 21th, 28th day).

2.9. Statistical analysis

The quality is described using the terms “average” and “standard deviation.” One path inspection of change (ANOVA) was used, along with Dunnett's “t” test, to identify the tangible influence. The p-value of 0.05 was deemed the significance level.

3. Results

3.1. Characterization of isolated compound

3.1.1. Physical characterization of Luteolin

Compound I: Luteolin was acquired as a yellow crystalline solid which provided a positive Shinoda test for flavonoid. The solubility of luteolin was found to be in an aqueous alkaline solution (Table 1, Fig. 1).

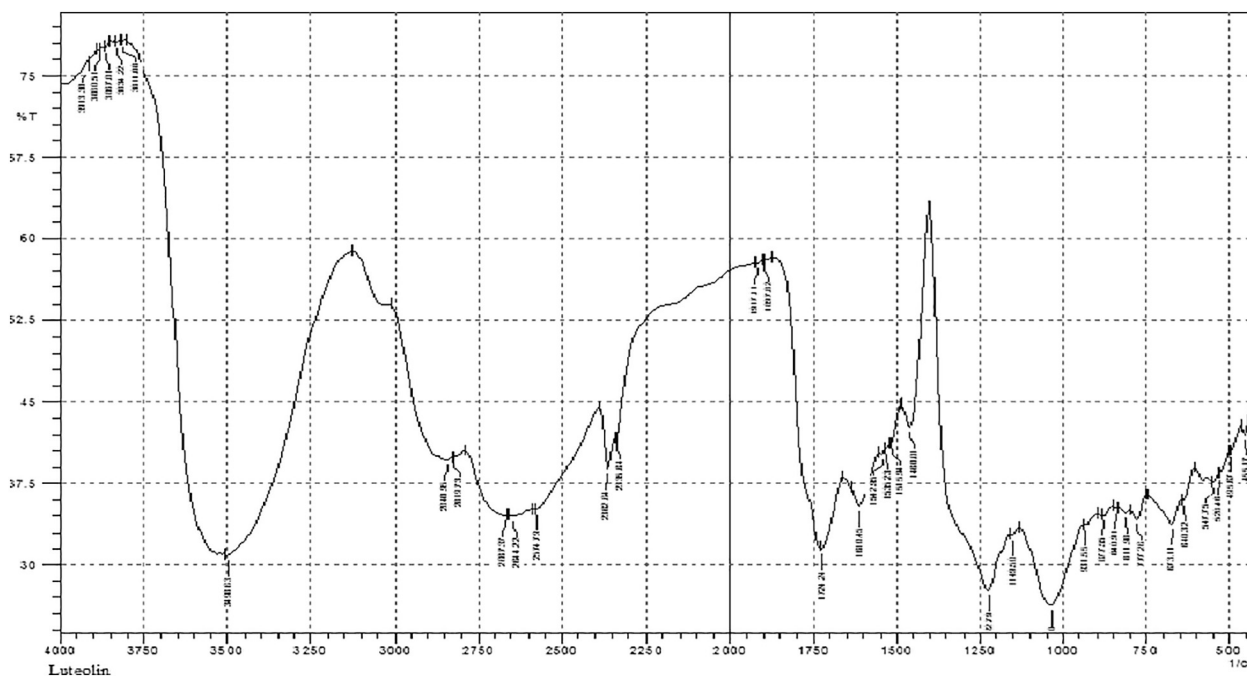


Fig. 1. IR Spectra of Luteolin.

3.2. IR spectra

3.3. ^1H NMR of Luteolin

^1H NMR (400 MHz, Chloroform) δ 7.00 (d, J = 13.5 Hz, 2H), 6.73 (s, 1H), 4.78 (s, 1H), 4.24 (s, 1H), 3.81 (s, 1H), (s, 1H), 6.56 (s, 1H), 6.18 (s, 1H), 6.05 0.40 (s, 1H) (Fig. 2).

Mass Spectra: The m/z value for luteolin was observed as 285 as shown in Fig. 3.

3.4. Anti-arthritis activity of Luteolin in FCA induced arthritis in rats

3.4.1. Effect of Luteolin on paw volume in FCA induced arthritis in rat
“Average” and “standard deviation” is used to judge the performance. To determine which impact was tangible, one path inspection of transformation (ANOVA) was used, together with Dunnet’s “t” test. It was considered significant if the p-value was below 0.05 (Table 2).

For each experimental group, the mean and standard error of the mean is provided. The SEM is 6 in each group. P-values are as follows: arthritic control < 0.05, arthritic control vs. control (un-

Table 1
IR spectra of Luteolin.

S NO.	Type of Bond appears	Reported cm^{-1} (Patora & Klimek 2002)	Observed cm^{-1}
1.	O-H (stretching alcohol)	3490	3498.90
2.	C-O (stretching alcohol)		
3.	C=O (Stretching carboxylic acid)	1616	1610.45
4.	O-H (Stretching carboxylic acid)	3114	
5.	C-H (stretching aromatic)	1470–1490	1468.04
6.	C=C (stretching aromatic)	1640	1643.24
7.	C-H (stretching aliphatic)	2950	2848

published data) < 0.01, and arthritic control vs. arthritic control (unpublished data) < 0.001.

3.5. Effect of Luteolin on arthritic score

The evidence of joint pain first became apparent after eight to ten days post-vaccination, and it reached its most severe level four weeks after that. Swelling, redness distortion, and contracture were apparent on the fourteenth day of the disease in the joints of the rear paws and lower legs. The manifestations of joint control rodents differed considerably from those of regular rodents with regards to the shape of their rear paws. This discovery has already been made: these manifestations were found to be less in the forelimbs. Even with just reasonable joint inflammation, cured ligament rodents proved redness and growth, and the ligament rodents given luteolin (both portions) revealed essentially no signs of joint inflammation. The impact was crucial because it provided a dramatic contrast to the ligament rodents Table 3:

3.6. Effects of Luteolin on body weight

Although the weights were almost identical in all groups of animals at 0 to 7 days during the subsequent course of the disease, the bodyweight always declined in the arthritic control group from the 14th day to the 21st day. In the arthritic group, a decrease in body weight was observed on the subsequent days, whereas groups treated with luteolin & betulinic acid showed improvements in body weight dose-dependently. This effect was observed from the 14th day to the last day of the experiment as compared to arthritic rats. Luteolin increases the bodyweight significantly as compared to the arthritic rats. ($P < 0.001$). The results are summarized in Table 4.

For each experimental group, the mean and standard error of the mean is provided. The SEM is 6 in each group. P-values are as follows: arthritic control < 0.05, arthritic control vs. control (unpublished data) < 0.01, and arthritic control vs. arthritic control (unpublished data) < 0.001.

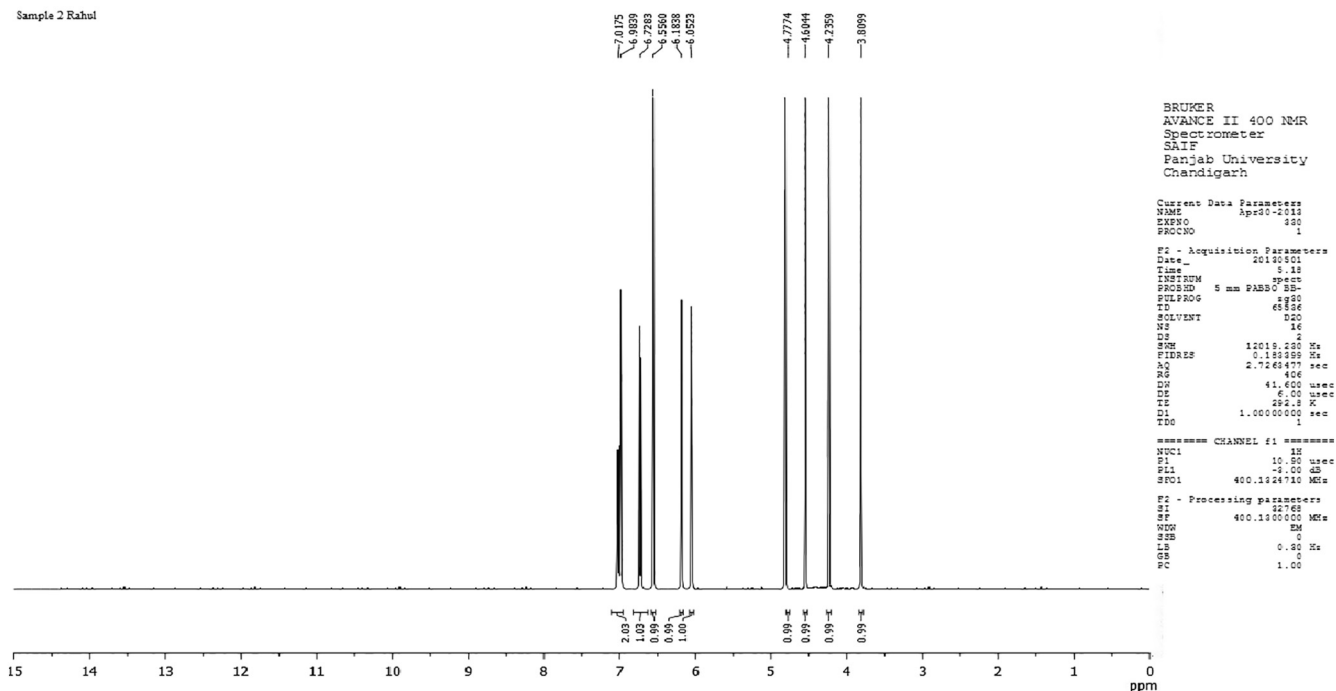
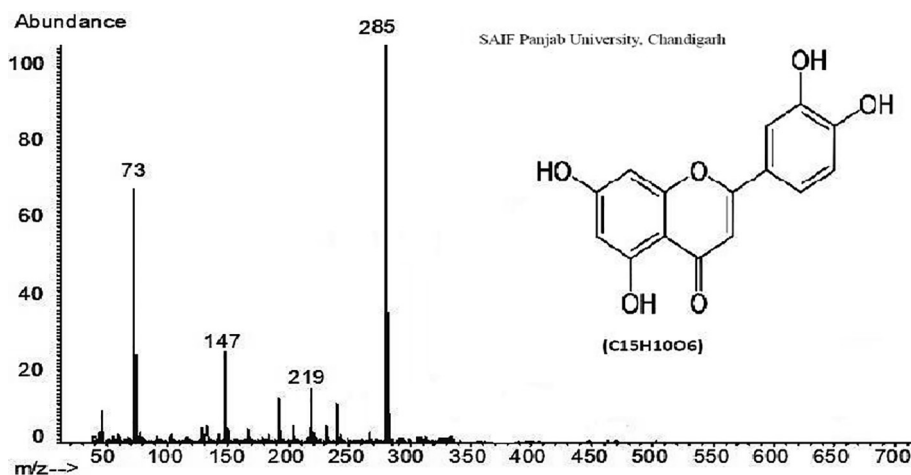
Fig. 2. ¹H NMR spectra of Luteolin.

Fig. 3. Mass spectra of Luteolin.

Table 2
Effect of Luteolin on Paw Volume.

S. No.	Groups & Treatments	Paw volume in (ml)				
		Zero Day	7th Day	14th Day	21st Day	28th Day
1	Normal Control	0.40 ± 0.05	0.39 ± 0.04	0.39 ± 0.02	0.40 ± 0.05	0.40 ± 0.06
2	Arthritic Control	0.40 ± 0.07	0.86 ± 0.13	1.50 ± 0.12	1.70 ± 0.07	1.95 ± 0.11***
3	Prednisolone 10 mg/kg	0.40 ± 0.06	0.82 ± 0.10	1.88 ± 0.14	0.90 ± 0.10	0.42 ± 0.11*** (78.46)
4	Luteolin (20 mg/kg)	0.39 ± 0.08	0.90 ± 0.12	1.90 ± 0.20	1.08 ± 0.20	0.52 ± 0.20** (73.33)
5	Luteolin (40 mg/kg)	0.41 ± 0.03	0.92 ± 0.10	1.92 ± 0.22	0.98 ± 0.22	0.45 ± 0.15*** (76.92)

4. Discussion

Polyphenols are auxiliary metabolites of plants ensnared in pigmentation, generation, and assurance against microorganisms. At present, there are in excess of 8000 known polyphenols sharing a

typical substance structure (hydroxyl bunch on the fragrant ring) with various constituents. Flavonoids are the most bountiful polyphenols present in the human eating routine and address a class of particles portrayed by a C6–C3–C6 spine structure. Natural products, vegetables, spices, and other plant food are all flavonoid

Table 3
the influence of Luteolin on an arthritic score.

S. No.	Groups & Treatment	7th day	14th day	21th day	28th day
1.	Arthritic Control	3.40 ± 0.15	3.54 ± 0.13	4.15 ± 0.20	4.52 ± 0.10
2.	Prednisolone (10 mg/kg)	3.28 ± 0.11	3.41 ± 0.10	2.22 ± 0.16***	1.25 ± 0.14***
3.	Luteolin (20 mg/kg)	3.25 ± 0.11	3.41 ± 0.15	3.20 ± 0.21***	2.20 ± 0.27***
4.	Luteolin (40 mg/kg)	3.32 ± 0.14	3.60 ± 0.10	2.90 ± 0.15***	1.89 ± 0.22***

Values are expressed as mean ± SEM, n = 6 in each group; *P < 0.05, compared to arthritic control **P < 0.01, compared to arthritic control. ***P < 0.001, compared to arthritic control.

Table 4
Effect of Luteolin on Body Weight.

S. No.	Groups & Treatments	Body Weight			
		Zero Day	14th Day	21st Day	28th Day
1	Normal Control	185.40 ± 2.34	188.15 ± 2.30	190.50 ± 2.12	193.20 ± 1.18
2	Arthritic Control	186.80 ± 2.40	160.50 ± 2.18	149.30 ± 1.20**	138.50 ± 2.30***
3	Prednisolone 10 mg/kg	180.40 ± 2.80	160.20 ± 2.10	185.40 ± 1.24**	197.30 ± 1.30***
4	Luteolin (20 mg/kg)	190.50 ± 3.10	173.80 ± 3.53	189.50 ± 2.80**	194.10 ± 2.17**
5	Luteolin (40 mg/kg)	192.30 ± 3.30	171.30 ± 3.10	196.82 ± 3.10***	201.50 ± 3.15***

sources. Flavonoids can be partitioned into a few subclasses as per various constituents, for example, flavanones, flavone, flavanols, and flavonols. It has been accounted for already that flavonoids have a wide scope of medical advantages due to their nutraceutical properties, for example, antibacterial, cancer prevention agent, and calming [8].

Flavonoids improve the incendiary reaction's impediment of provocative catalysts like responsive oxygen species (ROS) and nitric oxide (NO). Flavonoids contribute to the inhibition of such provoking catalysts as receptive oxygen species (ROS) and nitric oxide (NO). those by Lee et al. [6], and those by Maquart et al. [7]

Clinical and neurotic changes in humans with rheumatoid joint inflammation are very comparable to those produced in CFA-actuated joint pain. Worst case scenario (the CFA model getting worse) is represented as an enlargement of the paw infusion quantity.

A greater paw volume is in connection with the TNF- α and an increase in prostaglandin synthesis [5]. Prostaglandins are also known to participate in the third phase of inflammation-mediated vascular responses. TNF- α inhibition and prostaglandin synthesis inhibition may be linked to luteolin and betulinic acid's ability to reduce paw volume. The paw volume of arthritic rats in our research was reduced due to treatment with luteolin, as were the effects of these two compounds.

The Polyarthritic list is an immune response in which edema and thickening of the fragile tissue occur in the site as well as the exacerbation effect of the adjuvant, along with the sickness effect of the infused foot [12]. The administration of luteolin reduced the polyarthritic index in a significantly better manner as compared to rats with rheumatoid arthritis, and this result could be attributed to the treatment's ability to modulate the immune system.

During the growth of the symptoms, the weight gain of rats used for an indirect indicator was included. At the test sites and treatment groups, the body size of the rodents remained unchanged when compared with their usual weights. Researchers discovered that people who feel emotionally distressed are more likely to lose weight. As the incidence on the rodents rises with the accumulation of joint pain, so does the liability on the body [14]. Somasundaram et al. [11] found that incipient colitis causes rats to lose the ability to digest 14C-glucose and 14C-leucine, which may help to explain why calming drugs cause a reduction

in the rat's consumption of those two substances. In a study we conducted, we found that prednisolone, luteolin, and possibly the body's capacity to retain nutrients were associated with increasing body mass. Based on our findings, it appears that luteolin has the potential to act as an anti-arthritic compound.

Oxidative pressure has been expected to play a critical role in arthritis and joint pain in previous studies. It is a well-known fact that lipid peroxidation occurs during rheumatoid arthritis (RA), which is evidenced by measuring tissue MDA. Harm by free radicals appears to be the probable cause of a large amount of MDA in ligament control gathering [13].

5. Conclusion

It can be concluded that in long-term reductions in rat paw edema, arthritic index, and numerous lysosomal enzymes, Luteolin has health benefits. It showed no toxic effect on arthritic rat joints while showing a preventive role on joints with the disease. Since in acute inflammatory reactions, prostaglandin formulation is inhibited, as well as various lysosomal enzymes, cytokines production, and cytokine release, and in prolonged chronic inflammation, all of these processes are inhibited, there is merit to the point raised by Siddha and Ayurveda. Prednisolone was seen to be less effective when treating prostate cancer, decreasing the 78.46% effectiveness to 69.47%. It is worth mentioning that this statement can be concluded to be true: 'As a result, it may be stated that the Luteolin has long-term therapeutic benefits in rat paw edoema, arthritis index, and numerous different lysosomal enzymes.' To show that it also protects arthritic rat joints but without complications of toxicity, the results were verified by running experiments in rat knee joints. The evaluation of chronic inflammatory response was induced in rats by FCA (0.1 ml) injected intra-articularly in the left ankle joint on the 0th day.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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