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Chemical characterization and investigation of therapeutic properties of lemon peel supplementation for the management of induced gout arthritis by reducing xanthine oxidase in male albino rats

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Abstract: Gout arthritis is a joint pain disorder characterized by swelling around joints and elevated uric acid levels. These elevated uric acid levels result in joint stiffness and pain. The elevated uric acid level is due to over activity of xanthine oxidase. Lemon peel can inhibit the activity of xanthine oxidase. In this study, twenty male albino Wistar rats with induced arthritis were enrolled and were divided into four groups; control group (G_o) and treatment group G_1, G_2, and G_3. Treatment groups were given three different levels of lemon peel 150mg, 300mg and 450 mg per kg of body weight. Serum uric acid levels were measured before and after the trial. Chemical characterization and phytochemical analysis were also performed. The presence of 9.07±1.16% moisture in lemon peel. A total of 1.71±0.13% ash was present in lemon peel while 62.45±8.09% of carbohydrates were present in lemon peel. 4.04±0.08% crude protein, 9.87±1.63% crude fat, and 12.86±0.09% crude fiber wass present in lemon peel. Lemon peel held a significant amount of TPC and TFC depicted in fig. 1. Total phenolic content (TPC) was 1204±23.02 mg GAE/100g and total flavonoid content (TFC) was 471.98±11.74 mg QE/100g. The investigation had shown a significant ($p<0.05$) reduction in uric levels in response to lemon peel powder in all treatment groups. ANOVA test showed a significant reduction in uric acid levels when lemon peel was given to subjects.

Keywords: Lemon peel, arthritis, uric acid, xanthine, xanthine oxidase, phenolic content, flavonoids, mineral analysis, chemical composition, animal study, induction of arthritis.

INTRODUCTION

Gout arthritis is a chronic condition of synovial joints that is characterized as an autoimmune disorder and results in inflammation around joints. It also results in low to high-grade fever, stiffness, and swelling around joints. These symptoms worsen when the disease prolongs from weeks to months. Gout arthritis is often due to the over-activity of xanthine oxidase (McInnes & Schett, 2011). Xanthine oxidase is an enzyme that is involved in metabolizing purine. Xanthine oxidase is also involved in chronic tissue damage by mounting oxidative stress owing to free radical production in the body. Xanthine oxidase is involved in the conversion of xanthine to uric acid (Hille & Nishino, 1995). This uric acid upon rising to the normal value starts accumulating in the body and more particularly in ankle joints. This accumulation and crystallization of uric acid in joints lead to gout arthritis and gout. Inhibiting the xanthine oxidase helps is lowering the levels of xanthine conversion into uric acid and accumulation of uric acid as well. Allopurinol, to the day, is the only known inhibitor of xanthine oxidase (Harrison, 2004). However, allopurinol has many associated side effects including the development of nephropathy, chronic liver diseases, and initiation of allergic reactions. Due to all these side effects, researchers are investigating natural ways to inhibit xanthine oxidase. Lemon peel extract is one of the naturally existing remedies that can be helpful in reducing the xanthine oxidase activity. This ability of lemon peel is due to the presence of bioactive compounds including caffeic acid, eugenol, luteolin, quercetin, pyrogallic acid, salicylic acid, and p-coumaric acid. All these active compounds not only reduce the activity of xanthine oxidase (Zou *et al*., 2018). Thus, reducing the inflammation and other symptoms of arthritis and joint pain. Lemon peel not only reduces arthritis signs and symptoms but also reduces oxidative stress in the body by increasing the levels of antioxidants in the body (Zhang *et al*., 2017). This study is based on chemical characterization and bio evaluation of lemon peel supplementation for the management of arthritis. This study focuses on the determination of proximate analyses, phytochemical analyses, and mineral determination of lemon peel.

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MATERIALS AND METHODS

Collection of raw material
Lemons were purchased from the local market. They were first washed with tap water and then with distilled water. After drying, the lemon peel was removed. Removed lemon peel was dried under shade for 15 days and then ground to refine powder.

Identification of lemon Peel
The peel or rind refers to the entire skin, the colorful outer portion and the bitter white pith that lies right beneath it. The white pith is bitter and unpleasant, while the zest has the bright flavor of the fruit.

Chemical characterization of lemon peel
Lemon peel was subjected to various assays described as follows:

Proximate profile of lemon peel
Lemon peel was analyzed for the following proximate profile including moisture content, ash, carbohydrates, crude protein, crude fiber, and nitrogen-free extract (NFE) according to the AOAC method (Garcia-Amezquita *et al*., 2018).

Phytochemical analysis of lemon peel
Phytochemical analysis of lemon peel was investigated for the determination of total phenolic content (TPC) and total flavonoid content (TFC). The amount of TPC AND TFC were measured in ug of Gallic acid equivalent per ml and ug of Catechin equivalent per ml respectively (M'hiri *et al*., 2015).

Determination of minerals
For the determination of minerals, a 5g sample of lemon peel was taken in a 100 ml volumetric flask. In this flask 10ml of hydrochloric acid (HCL) was added and 100 ml volume was prepared by using distilled water. To remove the impurities volume was filtered. The mineral standard was prepared with hydrochloric acid and lanthanum concentrations. The sample was analyzed using atomic absorption spectrophotometer (Hernández *et al*., 2005).

Investigation of therapeutic potential of lemon peel against arthritis
Research Animals
28 male albino Wistar rats of weight 180-200g were purchased from the National Institute of Health (NIH), Islamabad. They were given *ad libitum* access to food and water. All animals were kept in 12 hours cycle of dark and light.

Induction of gout arthritis in rats
To induce arthritis, bovine collagen and complete Freund Adjuvant was mixed in equal ratio. The initial dose of 100ug/200uL was given in dermal tissues in the tail and then after 18 days, a booster dose was given to the rats (Zou *et al*., 2018).

Study duration
The study was conducted for 8 weeks. It started from 17th August 2022 and continued till 17th October 2022.

Treatment groups and treatment plan
G_o: Group I: Arthritis control group: No treatment was given
G_1: Group II: Arthritis treatment group; lemon peel powder 150mg/kg of body weight through the gauge
G_2: Group III: Arthritis treatment group; lemon peel powder 300mg/kg of body weight through the gauge
G_3: Group IV: Arthritis treatment group; lemon peel powder 450mg/kg of body weight through the gauge

Collection of blood samples
At the beginning and end of the trial blood samples of rats were drawn from the tail in sample collecting tubes.

Biochemical test
The uric acid level in serum was investigated before and at the end of the trial.

Ethical approval
Ethical approval was taken from the Institute of Biosafety Committee.

STATISTICAL ANALYSIS

Descriptive statistical analysis using a two-sample t-test a Completely Randomized Design (CRD) was carried out to investigate the level of significance ($p<0.05$). All statistical analyses are done with IBM SPSS Statistics 20 version (Larson, 2008).

RESULTS

Proximate analysis of lemon peel
The quality of raw material used is significantly influenced by the measurement of the proximate values. To evaluate the quality characteristics of lemon peel, proximate determination including moisture, ash, crude fat, crude protein, fiber, and nitrogen-free extract was evaluated. The presence of 9.07±1.16% moisture in lemon peel. A total of 1.71±0.13% ash was present in lemon peel while 62.45±8.09% of carbohydrates were present in lemon peel. 4.04±0.08% crude protein, 9.87±1.63% crude fat, and 12.86±0.09% crude fiber was present in lemon peel. Values of proximate analysis for lemon peel are shown in table 1.

Phytochemical characters of lemon peel
Lemon peel holds a significant amount of TPC and TFC depicted in fig. 1. Total phenolic content (TPC) was 1204±23.02 mg GAE/100g and total flavonoid content (TFC) was 471.98±11.74 mg QE/100g.

Table 1: Proximate Analysis of Lemon Peel.

Proximate analysis	Composition (%)
Moisture	9.07±1.16
Ash	1.71±0.13
Nitrogen Free Extract	62.45±8.09
Crude protein	4.04±0.08
Crude Fat	9.87±1.63
Crude fiber	12.86±0.09

Mineral analysis

Mineral analysis was performed via atomic absorption spectrophotometer for Fe, Zn, Cu, Mn and Se. Mean±S.D for iron (0.37±0.32) mg/100g, zinc (0.31±0.01) mg/100g, copper(0.04±0.002) mg/100g, manganese (0.06±0.001) in mg/100g and selenium (2.04±0.87) ug/100g were determined.

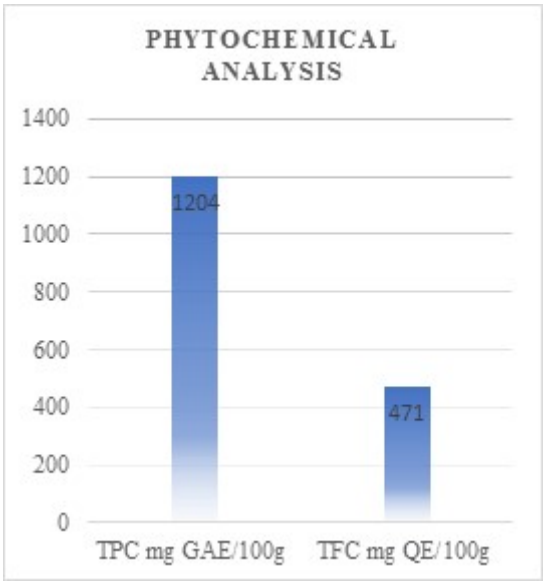

Fig. 1: Phytochemical analysis of lemon peel

Investigation of therapeutic potential of lemon peel against uric acid level for arthritis

The objective of this study was to investigate the medicinal effect of lemon peel supplementation for uric acid. Participants of the control group (G_0) were given no treatment while participants of treatment groups G_1, G_2, and G_3 were given an oral dose of lemon peel powder 150mg, 300mg, and 450mg to all treatment groups per kg of the body weight for 8 weeks. Before the commencement and after the standstill of the trial, blood samples were gathered from each subject for analysis of uric acid level.

Changes in uric acid level of rats

The investigation showed a significant ($p<0.05$) reduction in uric levels in response to lemon peel powder in all treatment groups. Treatment group G_1 showed a reduction in the serum uric acid level from 6.58±1.01mg/dl to 6.33±1.57mg/dl while treatment group G_2 showed a reduction in serum uric acid level from 6.67±1.24mg/dl to 6.23±2.12mg/dl. However, treatment group G_3 showed the most reduction in uric acid from 6.74±1.74 to 5.18±1.63 against the dose of 450mg/kg. However, the control group showed a slight increase in uric acid level levels.

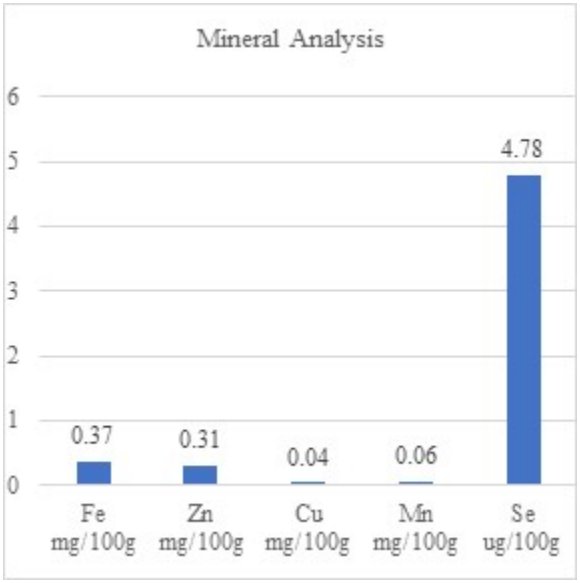

Fig. 2: Mineral analysis of lemon peel

DISCUSSION

Chemical characterization of lemon peel was done to evaluate the quality of the raw material used in the study. Proximate analysis showed that nitrogen-free extract i.e., carbohydrates make up around 60% proportion of the lemon peel. The rest of the constituents include moisture, ash, crude protein, crude fat, and crude fiber. The findings of this study are like the findings of other studies with minor differences. These minor differences can be due to variations in the region in which lemon is used and environmental conditions (Oikeh *et al*., 2013). One of the

Table 2: Mean ±S.D for serum uric acid level of rats in mg/dl

Duration	G_O	G_1	G_2	G_3	*p*-value
0 week	6.56±0.87	6.58±1.01	6.67±1.24	6.74±1.74	p<0.05
8th week	6.78±1.84	6.33±1.57	6.23±2.12	5.18±1.63	p<0.05

G_o: No treatment was given; G_1: Group II: lemon peel powder 150 mg/kg of body weight through gauge; G_2: Group III: lemon peel powder 300 mg/kg of body weight through gauge; G_3: Group IV: lemon peel powder 450 mg/kg of body weight through the gauge

most sensitive and dramatic indicators of gout is neutrophil influx into the joint fluid. Neutrophils accumulate in both the joint fluid and the synovial membrane, where a small fraction of these cells actively phagocytose monosodium urate crystals and release mediators, that are chemotactic and amplify the inflammatory reaction. The enzyme XO catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid, which plays a crucial role in gout. XO is an important source of oxygen derived free radicals. The enzyme catalyzes reduce oxygen (during reperfusion phase), leading to the formation of superoxide anion radicals and hydrogen peroxide, as well as hydroxyl radicals. It has been proposed as a central mechanism of oxidative injury in some situations like gout, ischemia, renal damage, hypertension, diabetes, etc. Recent findings show that the occurrence of gout is increasing worldwide, possibly due to the changes in dietary habits like intake of high-purine foods viz., organ meats, yeast, beer and other alcoholic beverages. The main therapeutic approach for gout is the use of XOI such as allopurinol, which block the final step in the synthesis of uric acid from purines. We investigated the effect of a lemon-peel extract on the levels of biochemical markers in a rat model of rheumatoid arthritis. Researcher reported that reduced cellular antioxidant and increased free radical levels are risk factors for rheumatoid arthritis. Lipid peroxyl radicals are the end products of membrane fatty-acid oxidation. Oxidative and inflammatory injuries increase prostaglandins in rheumatoid arthritis. Membrane-lipid per- oxidation is accelerated in rheumatoid arthritis. Researcher reported that MMP-3 produced by synovium-lining cells destroys type IX collagen and activates pro-collagenase.

An elevated serum MMP-3 level is indicative of cartilage and radiological damage. Researcher reported that ceruloplasmin and copper levels are increased in rheumatoid arthritis. Activation of xanthine oxidase increases the uric acid level in rheumatoid arthritis. The zinc level is increased in rheumatoid arthritis due to an increased level of IL-1. Pro-inflammatory factors such as ROS, PGE2, IL-6, IL-1β, and TNFα are related to the pathogenesis of rheumatoid arthritis. Chondrocytes and synovium-lining cells produce IL-1 and TNFα in the affected joints. Researchers have reported that the flavonoids (luteolin, quercetin, and chrysin) and polyphenol reduced the cardiovascular disease and obesity. Anti-inflammatory, anti-oxidant, anti-tumor and immunomodulatory effects of flavonoids have been reported.

Phytochemical analysis was also performed. In the study, total phenolic content and total flavonoids were also evaluated. The findings presented in fig. 1 showed the presence of a significant number of phytochemicals in the lemon peel. The presence of antioxidant compounds validates the antioxidant properties of lemon peel (Abd El-ghfar *et al.,* 2016). Chemical analysis showed the presence of minerals in the mentioned amounts in fig. 2. Analyses have shown the presence of Fe, Zn, Cu, Mn, and Se in the lemon peel. Se and Fe are antioxidant minerals, and all minerals are part of enzymes. These findings were consistent with the values of previously existing literature (Czech *et al*., 2020). Lemon peel supplementation showed a reduction in uric acid levels for all three doses of lemon peel powder. An oral dose of 450mg/ kg of the body weight showed the most reduction in uric acid levels. This ability of lemon peel is due to the presence of bioactive components of the lemon peel which inhibit the activity of xanthine oxidase. Thus reducing the uric acid accumulation in the joints. These findings were similar to the finding of another study (Zou *et al*., 2018, 2018).

CONCLUSION

Arthritis and gout are conditions of inflammation around joints due to the accumulation of uric acid crystals. Lemon peel has shown the ability to reduce uric acid levels by inhibiting the activity of xanthine oxidase. It also possesses antioxidant minerals including selenium and iron which play a role in reducing oxidative stress in the body. The reduction in oxidative stress helps lower the inflammation and inflammatory cytokines in the body. Lemon peel contains a generous amount of phytochemicals which further mount up the therapeutic potential of lemon peel.

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