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Full Length Article

# Anti-inflammatory potential of aloe vera meatballs and their impact on rheumatoid arthritis

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ABSTRACT

Rheumatoid Arthritis is an auto-immune condition resulting in the formation of autoantibodies that cause joint and systemic inflammation. Different signs and symptoms include pain, redness, and swelling in joints leading to difficulties performing day-to-day activities and restlessness. The prevalence of Rheumatoid Arthritis worldwide and in Pakistan is 0.5 % and 1 %, respectively, with females experiencing a higher ratio due to improper diet, weight loss, fatigue, and housework. Aloe vera chicken meatballs, rich in protein, calories, phenolic compounds, polysaccharides, and collagen protein, have anti-inflammatory effects on arthritis patients by reducing inflammatory mediator production. By considering this, we conducted an experiment on the production of Aloe Vera based chicken meatballs with different doses including (100 g of chicken, 10 g of Aloe Vera + 90 g of Chicken, 20 g of Aloe Vera + 80 g of Chicken, 30 g of Aloe Vera + 70 g of Chicken) at Meat Science Nutraceutical Laboratory, National Institute of Food Science and Technology, University of Agriculture, Faisalabad. 100 g of chicken was considered as a control treatment. The meatballs were tested on four different groups of rheumatoid patients at Allied Hospital Faisalabad. The results indicated that the combination (20 g of Aloe Vera + 80 g of Chicken) yielded the most significant reduction in the Arthritis Factor Test by 26 %, compared to untreated arthritis patients. Additionally, it showed 20 % higher acceptability than the control group. Therefore, meatballs containing 20 g of Aloe Vera and 80 g of Chicken were deemed the most effective biomedicine for alleviating rheumatoid arthritis in humans.

## 1. Introduction

Arthritis, derived from the Greek “arthro” and “itis”, is a medical term for swelling or joint inflammation, affecting connective tissues, joints, and surrounding tissues (Radu & Bungau, 2021). It has been documented since 4500 BC and is caused by factors like obesity, genetics, injuries, auto-immune disorders, muscle weakness, age, and wear and tear of joints (Aletaha & Smolen, 2018). 21 million US residents and 67 million expected to receive arthritis diagnoses by 2030, with 25 % of rural and 28 % of urban Pakistani populations suffering from osteoarthritis (Deane & Holers, 2019). JIA is a common childhood arthritis characterized by frequent uveitis, fatigue, and vision loss (Heiligenhaus et al., 2013; Martini et al., 2022; Stoll & Cron, 2014). Gout, inflammatory arthritis, requires urate-lowering treatments and patient analysis for risk factor identification. Pseudo-gout, a self-limiting condition, causes gout-like symptoms due to calcium pyrophosphate crystal

*Abbreviations:* RA, Rheumatoid Arthritis; JIA, Juvenile Idiopathic Arthritis; AFT, Arthritis Factor Test; RAFT, Rheumatoid Arthritis Factor Test; DMARDs, Disease Modifying Antirheumatic Drugs; TFC, Total Flavonoid Content; TPC, Total Phenolic Content; BMI, Body Mass Index; ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; G, Gram; BC, Before Christ; NIFSAT, National Institute of Food Science and Technology; UAF, University of Agriculture Faisalabad.

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deposition in joints (Ayub et al., 2023; Dang et al., 2021; Dehlin et al., 2020).

RA is an auto-immune condition characterized by joint inflammation, autoantibodies, systemic inflammation, and genetic and environmental factors. Common symptoms include sleep difficulties, arthritic nodules, inflamed joints, numbness, tingling, and burning sensations in the hands and feet (Deane & Holers, 2019), and cause disability (Zhang et al., 2024). The prevalence of rheumatoid arthritis worldwide and in Pakistan is 1 % and 0.5 % respectively (Lau, 2023). RA prevalence varies in genders, with women experiencing higher rates due to diet, weight loss, fatigue, and housework. Treatment with NSAIDs can cause kidney and hepatic failure (Intriago et al., 2019). Rheumatoid arthritis is a complex condition with early symptoms similar to other illnesses, making diagnosis challenging (Aletaha & Smolen, 2018). Treatments include aspirin, ibuprofen, corticosteroids, and DMARDs. Physical treatment, rest, posture, surgery, and orthopedic devices are used to reduce pain and swelling. Prognosis is unknow. The combination of ROS scavenging, on-demand CO gas release, and IL-6 signaling blockade results in potent therapeutic effect and synergistic immunomodulation impact, significantly alleviating the RA symptoms and preventing joint destruction (Zhang et al., 2024).

Aloe vera, originating from Africa, is a cactus-like plant with a sticky gel and reddish-yellow juice. With over 200 species, it's known for its bitter shining material (Joseph & Raj, 2010). Aloe vera, a medicinal plant, was utilized by ancient cultures like Egyptians, Arabians, Indians, Romans, and Greeks, and even by Alexandra The Great for wound healing of soldiers (Joseph & Raj, 2010). Aloe vera leaves are mostly water and contain over 200 chemicals, with carbohydrates being the largest component (Zhang et al., 2018). Aloe vera gel, containing proteins and lipids (Fig. 1), has a pH of 4–5 and contains cell walls, microparticles, organelles, and liquid gel. It contains phenolic compounds, amino acids, glycoproteins, enzymes, vitamins, and trace minerals (Rodríguez et al., 2010). Aloe vera's exudate and latex contain phenolic compounds, including anthraquinone, which contributes to its bitterness and anti-inflammatory properties, making it useful for laxatives (Minjares-Fuentes et al., 2018).

Aloe vera has anti-inflammatory, anti-diabetic, anti-tumor, and immunosuppressive properties. It aids in wound healing and burns and has been shown to prevent hyperglycemia in rats (Gao et al., 2019). Aloe vera is beneficial for type II diabetics, treating peptic ulcers, gastrointestinal tract infections, cancer, and aging, and is used as a national medicine for hypertension in Trinidad and Tobago. This gel is used to treat wounds and oral infections, relieve itching, and heal sores (Rodríguez et al., 2010). Aloe vera juice boosts health by increasing energy, and weight, and managing stress. It contains minerals, vitamins, and trace elements, treats constipation, regulates digestion, and aids weight loss (Grace, 2015).

Chickens have a long history, used for food in the Roman Empire, and various breeds emerged in the 19th century. Some of these breeds are still used today (Zaheer, 2015). Pakistan's chicken industry, established in 1960, meets protein demand and has rapidly grown due to its lean, high protein content (Fig. 1) and nutrient value (Hussain et al., 2015a). Pakistan's chicken meat industry experiences a 1.2 % growth rate annually, offering low-fat, nutrient-dense food with high iron and zinc content (Khan & Sameen, 2018). It is rich in vitamin B and has a low ultimate pH, making it acidic. However, homogeneity in meat quality is a concern for the industry, as it affects the overall nutritional value of chicken. High-temperature meat can denature protein, affect meatball color, and reduce nutritive value. Chicken meat is versatile and nutritious, used in barbecue and meatballs. Aloe vera chicken meatballs can be prepared by mixing aloe vera and minced chicken with spices.

The main objectives of this study with a clear hypothesis were to check the anti-inflammatory effect of Aloe Vera meatballs and to explore the influence of Aloe Vera meatballs on rheumatoid arthritis in human subjects.

## 2. Material and method

This examination was conducted in the Meat Science Nutraceutical Laboratory, a Hi-Tech food Analysis Laboratory situated at the National Institute of Food Science and Technology (NIFSAT) of the University of Agriculture Faisalabad (UAF). All the materials required, and systems followed were talked about below:

### 2.1. Procurement of raw material

After checking the maturity and size, fresh aloe vera *(Aloe barbadensis)* leaves were obtained from the botanical garden, a research area of UAF. Aloe vera was cleaned and risen three times with double-distilled water to remove dust and ionic particles. After that, aloe vera was trimmed and cut into pieces by using a sterilized knife. Gel was collected by removing the outer covering, followed by grinding by using a fast motor blender at 10000 rpm, and collected in sterilized jars containing a 95 % ethanol solution. On the other hand, a fresh chicken sample (breast) was obtained from native meat plants. The chicken was surface sterilized and trimmed to remove bloody veins and ionic particles. After that, the chicken was subjected to a blender to grind it at 10000 rpm and collected in sterilized jars.

### 2.2. Product development

The ground chicken meatballs were made manually and cooked for 5–10 min in a small quantity of oil using a vacuum frying machine at an internal temperature of 75 °C (Golge et al., 2018; Song et al., 2017). The

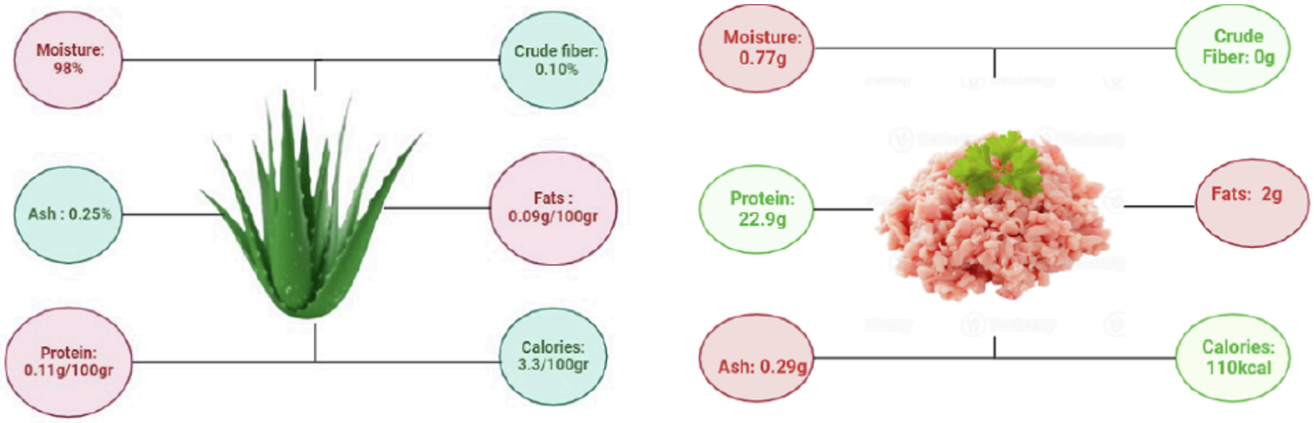


**Fig. 1.** Nutritional Profile of Aleo Vera Gel and Minced Chicken.

internal temperature was measured by inserting a temperature probe at a 2-minute interval. Aloe vera gel was carefully added after the cooking process of the chicken. The calculated quantity of both chicken and aloe vera is presented in Table 1. After 4–5 min, a quantity of salt, red chili powder, and turmeric powder were added for taste. The meatballs were placed in laminated nylon bags and stored in boxes at room temperature for further biochemical analysis.

### 2.3. Proximate analysis of product

#### 2.3.1. Moisture percentage

The (Paez et al., 2016) assessed the aloe vera chicken meatballs moisture content. To begin, the sample was weighed and placed in a hot air oven in an oven-dried China dish for 2 h at 105 °C. The dried China dish was removed from a hot air oven after 24 h and placed in a desiccator to avoid moisture.

$$\text{Moisture\%} = \frac{\text{Weight of original sample (g)} - \text{Weight of dried sample (g)}}{\text{Weight of original sample (g)}} \times 100$$

#### 2.3.2. Ash percentage

Aloe vera chicken meatballs' ash content was calculated using the (Paez et al., 2016).The aloe vera chicken meatballs sample was placed in a pre-weighed crucible and burned for 10–15 min on a Bunsen burner until the exhaust from the cauldron evaporated. Following the scorching and charring, the meatball sample was placed in a Muffle Furnace for direct burning at 550–600 °C until only white gruesome, gloomy, and miserable slag remained. After cooling in desiccators, the crucible was weighed. The equation below was used to determine the amount of ash (Marshall, 2010):

$$\text{Ash\%} = \frac{\text{Weight of ash in sample (g)}}{\text{Weight of original sample (g)}} \times 100$$

#### 2.3.3. Crude fat

The amount of fat in the aloe vera meatball was determined using the Gerber approach, which was then confirmed by the (Paez et al., 2016). The 10 ml $H_2SO_4$ solution was carefully measured using a pipette and placed into the butyrometer for fat estimation. Then combine 1 mL isoamyl alcohol with a 10.94 mL meat sample. The butyrometer was shaken for sample mixing after the stopper was carefully adjusted. After that, the sample was fed into the Gerber machine. The Gerber machine was set to 650 degrees Fahrenheit and 1100 revolutions per minute for 5 min. The butyrometer should be removed from the Gerber machine. The fat layer has been separated. The fat layer was measured on the butyrometer scale and compared to the graduation scale.

#### 2.3.4. Crude protein

Aloe vera chicken meatballs' crude protein content was evaluated using the (Paez et al., 2016). A clean digestive tube was obtained and filled with one digestion pill, 3 g of sample, and 30 ml of $H_2SO_4$. The sample was slowly cooked for 45 min to start the digestive process and prevent bubble formation, and then heated until it turned pale green. The digestion sample was placed to cool once the digestion was done. A 250 mL volumetric flask was used, which was cleaned 2–3 times with distilled water before the digested sample was transferred to it. Distilled water was added to the flask to bring the mixture's volume to 250 ml. In contrast, the ammonia produced in the distillation unit was gathered in a beaker with a solution of 4 % boric acid and a few drops of methyl red for identification. The reaction and procedure came to an end when the red became yellow. Titration of distilled material against 0.1 N sulfuric acid with a pink hue as the endpoint was the final step. The amount of sulfuric acid used was recorded. To quantify total protein, the following formula was used to compute total nitrogen content, which was then multiplied by a factor(N. Thiex, 2009).

$$\text{Nitrogen\%} = \frac{H_2SO_4(\text{ml}) \times 250 \times 0.0014}{\text{Weight sample} \times \text{Sample volume(digested)}} \times 100$$

$$\text{Total protein \%} = \text{Nitrogen \%} \times 6.38$$

**Table 1**
Quantity of dose for a particular treatment.

| Treatments | Dose |
|---|---|
| $T_0$ | Controlled group (100 g chicken) |
| $T_1$ | Aloe vera chicken balls (10 g aloe + 90 g chicken) |
| $T_2$ | Aloe vera chicken balls (20 g aloe + 80 g chicken) |
| $T_3$ | Aloe vera chicken balls (30 g aloe + 70 g chicken) |

### 2.4. Total flavonoid contents (TFC)

The total flavonoid content of the aloe vera meatballs was ascertained using the technique outlined by (Shad et al., 2013). 0.5 mL of meatball, 0.15 mL of distilled water, and a 5 percent $NaNO_2$ solution were mixed and incubated for 6 min. Subsequently, 0.15 mL of a 10 % AlCl3 solution was added, and the mixture was left to stand for an additional 6 min before adding 4 % NaOH solution. To make the reaction mixture a volume of 5 ml, methanol was added and well mixed. The absorbance of the reaction mixture was measured at 510 nm following a 15-minute incubation period. (M. A. Ayub et al., 2017). The meatballs' total flavonoid content (TFC) was calculated using the catechin linear regression curve and expressed as μg of equivalent catechin per milliliter of plant extract.

### 2.5. Determination of total phenolic contents (TPC)

According to (Saeed et al., 2019), the Folin-Ciocalteu method was utilized to ascertain the aloe vera meatballs' total phenolic content. With varying amounts of gallic acid, the calibration curve was created. The gallic acid solution in methanol at concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.10 mg/mL was combined with 5 mL of tenfold diluted Folin-Ciocalteu reagent and 4 mL of sodium carbonate (20 percent). After recording the absorbance at 765 nm for an hour, the calibration curve was drawn using the absorbance as a function of concentration. The same reagent as stated above was combined with 1 mL of plant extract (0.001 g/mL), and after an hour the absorbance of the resulting blue color complex was measured at 765 nm. Each determination was made three times.

$$\mathbf{T = C \times V/M}$$

Where,

T = total contents of phenolic compound in mg GAE/g meatball.

C = the concentration of gallic acid calculated from the calibration curve in mg/mL.

V = the volume of meatballs in mL.

M = the weight of meatballs in grams (g).

### 2.6. Human model and sensory evaluation

In human experimental modeling, 12 subjects were selected from different areas of Faisalabad, Pakistan, and kept under observation at the orthopedic unit of Allied Hospital Faisalabad. For efficacy trials, 4 groups were made having 3 individuals in each group as a replication. The sample was collected before treatment and after 30 days. The rheumatoid arthritis factor of subjects was checked by laboratory test as shown in Table 4. Using the (Tobin et al., 2013) described 9-point hedonic scale technique, sensory evaluation of many aspects including appearance, texture, taste, flavor, and overall acceptability was undertaken.

## 3. Statistical analysis

Analysis of variance (ANOVA) was used to establish the significance threshold for each parameter at the 5 % level of significance. ANOVA is a statistical method used to determine if there are significant differences between the means of three or more independent groups. It operates by comparing the variance within groups to the variance between groups, producing a p-value that indicates the significance of the observed differences. For this purpose, statistical software "Statistics//" version 8.1 was used for the Tukey HSD comparison test. It helps identify which specific groups' means are significantly different from each other. Values obtained were arranged in three groups which were significant, highly significant, and non-significant.

## 4. Results

### 4.1. Proximate analysis of aloe vera chicken meatballs

#### 4.1.1. Moisture content

The overall level of moisture in this investigation ranged from 61 to 67 % as shown in Fig. 2. The treatment $T_3$ had the highest moisture content 10 % (67 % vs 61 %) as compared to the control; however, the variation among treatments was statistically significant ($p < 0.02$).

#### 4.1.2. Ash content

In the $T_0$ and $T_3$ samples, the aloe vera chicken meatballs included 3.87 to 1.89 % ash content. The ANOVA results indicate a significant difference ($p < 0.024$), indicating the use of a mean comparison test to identify the most significant therapy; however, the variation among $T_0$ and $T_1$ was statistically non-significant as shown in Fig. 3.

#### 4.1.3. Fat content

The aloe vera chicken meatballs' fat content ranges from 15 to 12 %. The ANOVA results show a ($p < 0.029$), below 0.05, indicating statistical significance. A mean comparison test reveals that $T_0$ has the highest fat concentration among all treatments, indicating the significance of the findings. Notably, variation among $T_2$ and $T_3$ was statistically non-significant as shown in Fig. 4.

#### 4.1.4. Protein content

Protein levels in $T_0$ are 12 % and in the $T_3$ sample are 9 %. From $T_0$ to $T_3$, the value of protein content drops. The results of the ANOVA indicated that the ($p < 0.03$). Given that the p-value is less than 0.05, our findings are statistically significant; however, the variation among $T_2$ and $T_3$ was statistically non-significant. According to the mean comparison test, protein value decreases from $T_0$ to increasing treatment doses as shown in Fig. 5.

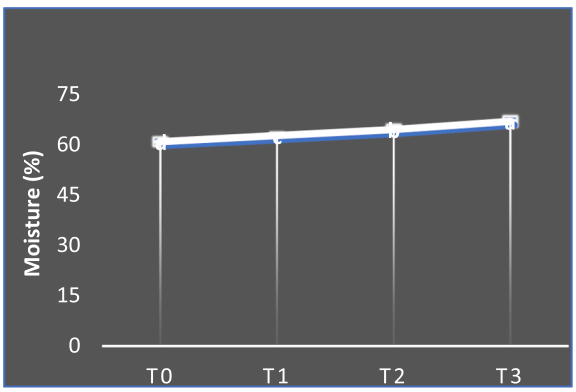


**Fig. 2.** Mean comparison of moisture.

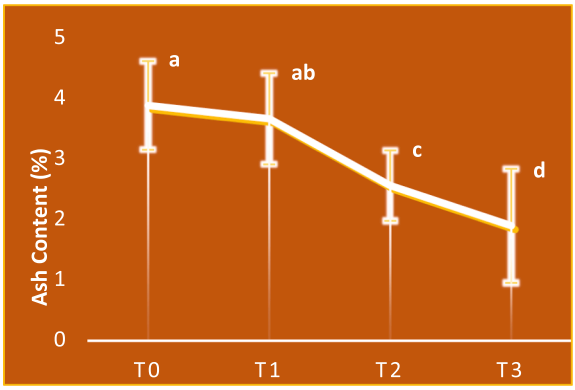


**Fig. 3.** Mean comparison of ash content.

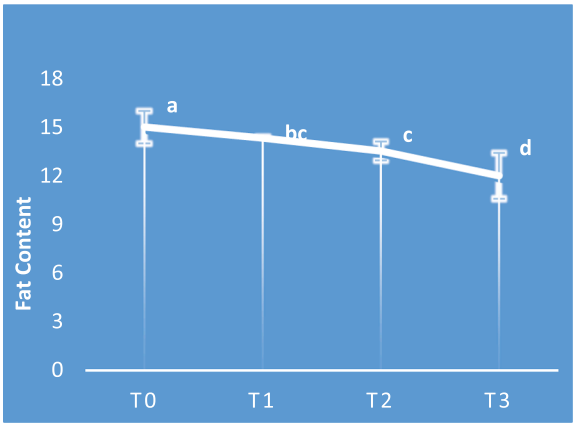


**Fig. 4.** Mean comparison of fat content.

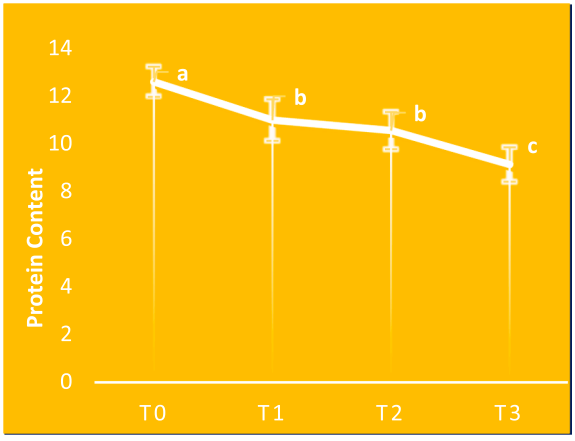


**Fig. 5.** Mean comparison of protein content.

#### 4.1.5. Crude fiber

A mean comparison test was used to check which therapy is non-significant from others. The value of all treatments indicates that $T_3$ has the highest concentration of fiber ($p < 0.02$). There was no detection of crude fiber in $T_0$ as shown in Fig. 6.

#### 4.1.6. TPC content

ANOVA of TPC content showed ($p < 0.04$), indicating that our results are significant. The mean comparison test is used to check which treatment is significant as TPC was increased by increasing treatment doses as shown in Fig. 7.

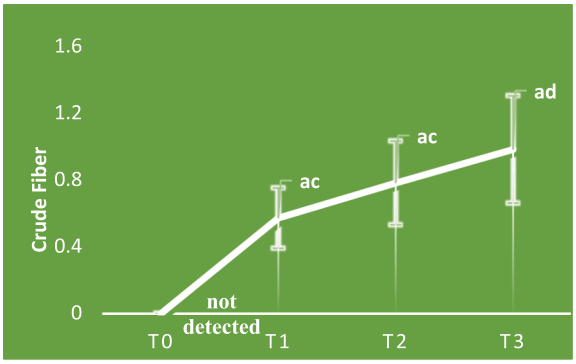


**Fig. 6.** Mean comparison of crude fiber.

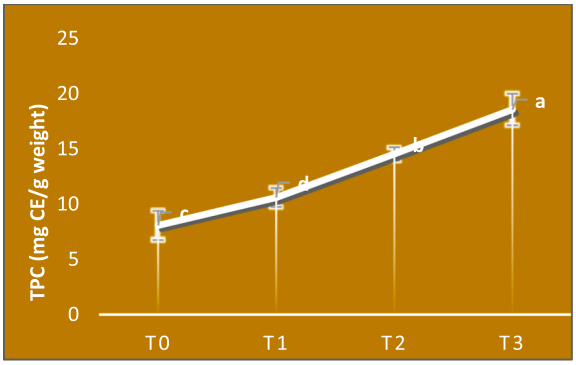


**Fig. 7.** Mean comparison of total phenolic content.

#### 4.1.7. Total flavonoid content (TFC)

It was discovered that the aloe vera chicken meatballs had a total flavonoid content of 12.55–13 mg CE/g weight. ANOVA shows a ($p < 0.02$), demonstrating the significance of our findings. However, the variation among $T_2$ and $T_3$ was statistically non-significant as shown in Fig. 8. The mean comparison test showed that the value of all treatments showing the value of TFC increases from $T_0$ to $T_3$.

### 4.2. Overall acceptability/ sensory evaluation

Aloe Vera chicken meatballs were made with 10 %, 20 %, and 30 % aloe vera gel, respectively. The panelists thought sample $T_2$ was generally acceptable. Meatballs were given a 20 percent aloe vera treatment, which exhibited substantial consequences in contrast to 10 % $T_1$ and 30 % $T_3$. According to the results, adding average aloe vera gel to meatballs improved their overall nutritional profile and meatball quality. The notable improvements are presented in Table 2.

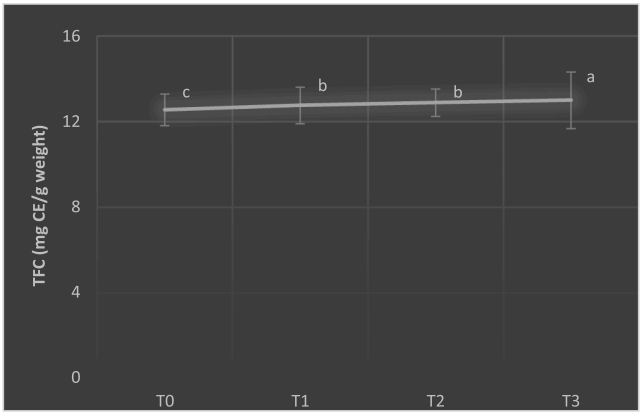


**Fig. 8.** Mean comparison of total flavonoid content.

### 4.3. Anthropometric measurement

BMI as shown in Table 3 of subjects was calculated by dividing weight in kg and height in m2. There are different ranges of BMI below 14 is under weight, 14–24.5 is considered healthy, 25–29.5 is overweight and above 30 is obese.

### 4.4. Biochemical assessment

The biochemical assessment was done at the start of 0th day and end of the study on 30th day. The biochemical assessment includes blood sampling known as rheumatoid arthritis factor RAF.

### 4.5. Rheumatoid arthritis factor test

The normal range of RAF is < 14 IU/ml. Subjects with RAF greater than 14 IU/ml have positive rheumatoid arthritis. The results showed very little difference in $T_3$ while the major difference was observed in the $T_2$ group because aloe vera showed dose-dependent characteristics.

## 5. Discussion

Arthritis, a major disability caused by obesity, family history, accidents, autoimmune issues, and aging, affects joints, tissues, and connective tissues, causing stiffness, pain, and physical signs. Rheumatoid arthritis is an auto-immune condition causing joint and systemic inflammation, autoantibodies, and sleep issues, diagnosed through the rheumatoid factor test. Aloe vera, an African plant, is used medicinally and as a food source due to its anti-inflammatory properties. Chicken, a key food source in Pakistan, has been used for centuries for treating diseases like obesity, diabetes, and ulcers. We hypothesized that Aloe Vera-based chicken meatballs could help reduce inflammation in rheumatoid arthritis as presented in the above parameters.

Meatballs' shelf life is significantly influenced by their moisture content, which is prone to microbial growth. Aloe vera chicken meatballs showed a significant increase in moisture content when aloe vera gel was added to a meatball containing 98 % water as shown in Fig. 2, indicating a higher moisture concentration in the $T_3$ sample. $T_0$ showed the highest ash concentration as shown in Fig. 3, indicating food items are not mineral-rich, and high ash content in meatballs shortens shelf life due to microorganisms requiring minerals. $T_0$ has the highest fat concentration, playing a crucial role in food sample quality and shelf life. Fats provide too much energy compared to other nutrients, with aloe vera chicken meatballs ranging from 14.96 to 12 percent as shown in Fig. 4. Protein content decreases from $T_0$ to T3 as shown in Fig. 5, a period when amino acids are chemically linked together to represent features and qualities. Aloe vera chicken meatballs contain various proteins, including collagen type II and C-glucosyl chromone, with varying concentrations. Protein, fat, and ash percentages were within the ranges reported by various workers (Rodríguez et al., 2010).The mean comparison test showed that $T_3$ has the highest fiber content,

**Table 2**
Mean Comparison of different sensory parameters of aloe vera chicken meatballs.

| Treatment | Taste | Texture | Aroma | Color | Acceptability |
|---|---|---|---|---|---|
| $T_0$ | 6.4 ± 0.48a | 8 ± 0.48c | 6.6 ± 0.15b | 5.9 ± 2.21c | 7 ± 0.290b |
| T2 | 6.6 ± 0.42b | 8.2 ± 0.51b | 7.1 ± 0.23ab | 6.8 ± 0.26b | 8.2 ± 0.14ab |
| T3 | 8.2 ± 0.58bc | 8.5 ± 0.21a | 7.2 ± 0.13a | 6.3 ± 0.28bc | 8.4 ± 0.33a |
| T4 | 5.4 ± 0.68C | 7.6 ± 0.26d | 7 ± 0.26ab | 7.5 ± 0.13a | 6.8 ± 0.23c |

**Table 3**
Mean comparison of BMI of rheumatoid arthritis subjects.

| Treatment | Mean |
|---|---|
| $T_0$ | 17.67c |
| $T_1$ | 27.67a |
| $T_2$ | 21ac |
| $T_3$ | 26ab |

**Table 4**
Blood samples of different subjects before and after treatment.

| Treatment | Blood sample before Treatment | Blood sample after Treatment |
|---|---|---|
| **$T_0$** | 19.7 ± 0.30a | 17.2 ± 0.27ab |
| **T2** | 18.9 ± 0.59ab | 16.8 ± 0.25b |
| **T3** | 18.7 ± 0.50ab | 13.8 ± 0.28c |
| **T4** | 18.5 ± 0.10b | 18 ± 0.36a |

while Aloe vera chicken meatballs contain 0 to 0.98 percent crude fiber shown in Fig. 6, primarily cellulose, derived from plant materials and weaned cellulose, an insoluble byproduct of acid and alkaline hydrolysis, this is equivalent to (Dhara et al., 2021; Mendy et al., 2019). Studies have revealed a linear link between radical scavenging ability and total phenolic content. The total phenolic and flavonoid contents of the meatballs significantly increased with increasing aloe vera concentration, with $T_3$ aloe vera chicken meatballs having the highest values as shown in Fig. 7 & Fig. 8, which is comparable to (Mahajan et al., 2021; Mendy et al., 2019). There have been claims that food products containing aloe vera gel and other plant extracts have antioxidant properties. The rheumatoid arthritis factor (RAF) is a test used to determine whether arthritis is positive or negative. Our study found that the anti-inflammatory effect of aloe vera chicken meatballs was dose-dependent, as mentioned by (Hussain et al., 2015b). The group receiving $T_2$ (20 g aloe and 80 g chicken) showed the maximum reduction in RAF (>14 IU/ml), comparable to the control group ($T_0$). This suggested that consumption of an average amount of aloe vera and chicken meatballs resulted in a maximum reduction in inflammatory mediators and a reduction in signs of arthritis. The panelists found sample $T_2$ acceptable, but meatballs treated with 20 % aloe vera showed significantly improved nutritional profile and quality compared to 10 % and 30 % treatments. The study highlights the potential of aloe vera gel in meatball production. This study is supported by a previous study conducted by (Guha et al., 2014; Paul et al., 2014).

## 6. Conclusion

Our data documents that Aloe vera Chicken meatballs can reduce inflammatory pain very efficiently if consumed daily and have high acceptability among participants with RA. Participants consuming aloe vera meatballs showed significant reductions in RA factor, IL-6, and TNF-α levels. This suggested that taking chicken meatballs with aloe vera in moderation led to the greatest decrease in inflammatory mediators and arthritis symptoms.

## 7. Study's limitations and future research Prospects

### 7.1. Limitations

- The intervention period of 30 days may not be sufficient to fully assess long-term effects of aloe vera-enriched meatballs on rheumatoid arthritis (RA) progression or symptom management.
- Cultural preferences and individual taste variability could influence the acceptability of aloe vera-enriched meatballs, potentially limiting their global applicability.

### 7.2. Future research prospects

- Conducting multicenter, randomized controlled trials with a larger and more diverse population is essential to confirm the anti-inflammatory benefits of aloe vera meatballs.
- Long-term studies are necessary to evaluate sustained effects on RA symptoms, disease progression, and overall health outcomes.
- Future research should focus on elucidating the molecular mechanisms by which aloe vera bioactives modulate inflammatory pathways and immune responses.
- Investigate the incorporation of aloe vera into other functional foods, such as beverages, baked goods, or vegetarian alternatives, to cater to a wider range of dietary preferences.
- Exploring how individual factors, such as genetic predispositions or gut microbiome composition, influence the effectiveness of aloe vera-enriched diets in managing RA.

By addressing these limitations and pursuing these research directions, future studies can provide a more robust understanding of aloe vera's role as a functional food in managing rheumatoid arthritis.

*CRediT authorship contribution statement*

**Warda Khan:** Methodology, Investigation, Conceptualization. **Rizwan Shaukat:** Supervision, Conceptualization. **Awais Khan:** Project administration, Methodology. **Amna Khan:** Validation, Investigation. **Bushra Ahmad:** Writing – original draft, Data curation. **Saira Saleem:** Writing – review & editing, Software, Formal analysis. **Mohammad Abul Farah:** Resources, Methodology. **Waqas Amin:** Software, Formal analysis. **Obaid Ullah Khan:** Writing – review & editing, Data curation.

## 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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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2024.103573.

## References

Aletaha, D., Smolen, J.S., 2018. Diagnosis and management of rheumatoid arthritis: a review. J. Am. Med. Assoc. 320 (13), 1360–1372.
Ayub, B., Frare, D., Romagnolli, L., Zanovelo, E.M., Maniglia, J.V., Maniglia, M.P., 2023. Calcium pyrophosphate dihydrate crystal deposition disease (tophaceus pseudogout) of the temporomandibular joint: a case report. J. Oral Maxillofac. Surg. Med. Pathol. 35 (3), 219–223.
Ayub, M.A., Hussain, A.I., Hanif, M.A., Chatha, S.A.S., Kamal, G.M., Shahid, M., Janneh, O., 2017. Variation in phenolic profile, β-Carotene and flavonoid contents, biological activities of two Tagetes species from Pakistani flora. Chem. Biodivers. 14 (6), e1600463.
Dang, R.R., Noonan, V., Chigurupati, R., Henry, A., 2021. Treatment of tophaceous pseudogout in the temporomandibular joint with resection and alloplastic reconstruction: a single-staged approach. Oral Maxillofac. Surg. 1–5.
Deane, K.D., Holers, V.M., 2019. The natural history of rheumatoid arthritis. Clin. Ther. 41 (7), 1256–1269.
Dehlin, M., Jacobsson, L., Roddy, E., 2020. Global epidemiology of gout: prevalence, incidence, treatment patterns and risk factors. Nat. Rev. Rheumatol. 16 (7), 380–390.
Dhara, D., Biswas, S., Biswas, O., Patra, G., Kandasawamy, P., 2021. Effect of pomegranate peel powder on cooked spent layers chicken meatballs. Indian J. Anim. Health 60 (2), 231–243.

Gao, Y., Kuok, K.I., Jin, Y., Wang, R., 2019. Biomedical applications of Aloe vera. Crit. Rev. Food Sci. Nutr. 59 (sup1), S244–S256.
Golge, O., Kilincceker, O., Koluman, A., 2018. Effects of different fibers on the quality of chicken meatballs. J. Food Saf. Food Qual. 69 (6), 177–183.
Grace, D., 2015. Food safety in low and middle income countries. Int. J. Environ. Res. Public Health 12 (9), 10490–10507.
Guha, P., Paul, S., Das, A., Halder, B., Bhattacharjee, S., Chaudhuri, T.K., 2014. Analyses of human and rat clinical parameters in rheumatoid arthritis raise the possibility of use of crude Aloe vera gel in disease amelioration. Immunome Res. 10 (2), 1–7.
Heiligenhaus, A., Heinz, C., Edelsten, C., Kotaniemi, K., Minden, K., 2013. Review for disease of the year: epidemiology of juvenile idiopathic arthritis and its associated uveitis: the probable risk factors. Ocul. Immunol. Inflamm. 21 (3), 180–191.
Hussain, S.A., Panjagari, N.R., Singh, R.R.B., Patil, G.R., 2015b. Potential herbs and herbal nutraceuticals: food applications and their interactions with food components. Crit. Rev. Food Sci. Nutr. 55 (1), 94–122.
Hussain, J., Rabbani, I., Aslam, S., Ahmad, H.A., 2015a. An overview of poultry industry in Pakistan. World's Poult. Sci. J. 71 (4), 689–700.
Intriago, M., Maldonado, G., Cárdenas, J., Ríos, C., 2019. Clinical characteristics in patients with rheumatoid arthritis: differences between genders. Sci. World J.
Joseph, B., Raj, S.J., 2010. Pharmacognostic and phytochemical properties of Aloe vera linn an overview. Int. J. Pharm. Sci. Rev. Res. 4 (2), 106–110.
Khan, M.I., Sameen, A., 2018. Animal Sourced Foods for Developing Economies: Preservation, Nutrition, and Safety. CRC Press.
Lau, C.S., 2023. Burden of rheumatoid arthritis and forecasted prevalence to 2050. Lancet Rheumatol. 5 (10), e567–e568. https://doi.org/10.1016/s2665-9913(23)00240-0.
Mahajan, K., Kumar, S., Bhat, Z.F., Naqvi, Z., Mungure, T.E., Bekhit, A.-E.-D.-A., 2021. Functionalization of carrageenan based edible film using Aloe vera for improved lipid oxidative and microbial stability of frozen dairy products. Food Biosci. 43, 101336.
Marshall, M.R., 2010. Ash analysis. Food Anal. 4, 105–116.
Martini, A., Lovell, D.J., Albani, S., Brunner, H.I., Hyrich, K.L., Thompson, S.D., Ruperto, N., 2022. Juvenile idiopathic arthritis. Nat. Rev. Dis. Primers 8 (1), 5.
Mendy, T.K., Misran, A., Mahmud, T.M.M., Ismail, S.I., 2019. Application of Aloe vera coating delays ripening and extend the shelf life of papaya fruit. ScientiaHorticulturae 246, 769–776.
Minjares-Fuentes, R., Femenia, A., Comas-Serra, F., Rodríguez-González, V.M., 2018. Compositional and structural features of the main bioactive polysaccharides present in the Aloe vera plant. J. AOAC Int. 101 (6), 1711–1719.
Paez, V., Barrett, W.B., Deng, X., Diaz-Amigo, C., Fiedler, K., Fuerer, C., Hostetler, G.L., Johnson, P., Joseph, G., Konings, E.J.M., 2016. AOAC SMPR® 2016.002. J. AOAC Int. 99 (4), 1122–1124.
Paul, S., Dutta, S., Chaudhuri, T.K., Bhattacharjee, S., 2014. Anti-inflammatory and protective properties of Aloe vera leaf crude gel in carrageenan induced acute inflammatory rat models. Int. J. Pharm. Pharm. Sci. 6 (9), 368–371.
Radu, A.-F., Bungau, S.G., 2021. Management of rheumatoid arthritis: an overview. Cells 10 (11), 2857.
Rodríguez, E.R., Martín, J.D., Romero, C.D., 2010. Aloe vera as a functional ingredient in foods. Crit. Rev. Food Sci. Nutr. 50 (4), 305–326.
Saeed, A., Marwat, M.S., Shah, A.H., Naz, R., Abidin, S.-Z.-U., Akbar, S., Khan, R., Navid, M.T., Saeed, A., 2019. Assessment of total phenolic and flavonoid contents of selected fruits and vegetables. Indian J. Tradit. Knowl. (IJTK) 18 (4), 686–693.
Shad, M.A., Nawaz, H., Rehman, T., Ikram, N., 2013. Determination of some biochemicals, phytochemicals and antioxidant properties of different parts of *Cichorium intybus* L.: a comparative study. J. Anim. Plant Sci. 23 (4), 1060–1066.
Song, L., Gao, T., Ma, R., Jiang, Y., Zhang, L., Li, J., Zhang, X., Gao, F., Zhou, G., 2017. Effect of different frozen storage temperatures and periods on the quality of chicken meatballs. J. Food Process. Preserv. 41 (1), e13042.
Stoll, M.L., Cron, R.Q., 2014. Treatment of juvenile idiopathic arthritis: a revolution in care. Pediatr. Rheumatol. 12, 1–10.
Thiex, N., 2009. Evaluation of analytical methods for the determination of moisture, crude protein, crude fat, and crude fiber in distillers dried grains with solubles. J. AOAC Int. 92 (1), 61–73.
Tobin, R., Moane, S., Larkin, T., 2013. Sensory evaluation of organic and conventional fruits and vegetables available to Irish consumers. Int. J. Food Sci. Technol. 48 (1), 157–162.
Zaheer, K., 2015. An updated review on chicken eggs: production, consumption, management aspects and nutritional benefits to human health. Food Nutr. Sci. 6 (13), 1208.
Zhang, Y., Bao, Z., Ye, X., Xie, Z., He, K., Mergens, B., Li, W., Yatcilla, M., Zheng, Q., 2018. Chemical investigation of major constituents in Aloe vera leaves and several commercial Aloe juice powders. J. AOAC Int. 101 (6), 1741–1751.
Zhang, Y., Liu, D., Chen, W., Tao, Y., Li, W., Qi, J., 2024. Microenvironment-activatable probe for precise NIR-II monitoring and synergistic immunotherapy in rheumatoid arthritis. Adv. Mater., 2409661