ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 01 | January - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IN VIVO ANTIOXIDANT POTENTIAL OF MILLETS IN ADJUVANT-INDUCED ARTHRITIS

Biochemistry

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ABSTRACT

Millets are Nutri cereals rich in bioactive compounds capable of inhibiting various diseases caused by lipid peroxidation. The aim of the present study was to determine the antioxidant potential of finger millet kodo millet and foxtail millet in arthritic rat models. Female Wistar rats were divided into six groups as Group I- control, Group II- adjuvant-induced arthritis rat (AIA), Group III to V - AIA groups supplemented with finger millet (FM) kodo millet (KM) foxtail millet (FoM) respectively. Group VI- AIA + indomethacin (IND) treated group. After 21 days of experiment, it was observed that the lipid peroxidation product malondialdehyde (MDA) was increased in AIA treated groups and after millet supplementation its concentration was significantly reduced. The activities of antioxidant enzyme, SOD and non-enzymatic antioxidant GSH were also measured in different tissues of arthritic rats. It is observed that millet supplementation improved the antioxidant status in different groups and that was comparable with standard drug. The current study showed the preventive effect of millets in arthritis progression by maintaining the antioxidant level in the system.

KEYWORDS

Millets, Oxidative stress, Malondialdehyde, Superoxide dismutase, Glutathione

INTRODUCTION

Rheumatoid arthritis (RA) is a complex multifactorial autoimmune disease affecting approximately 1% population in the world and prevalent among women which can almost affect all the organ system in the body [1]. Non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), analgesics etc. are mainly used as treatment option to reduce inflammation and pain but its regular consumption cause adverse side effects to the patients [2]. Once the disease progress, oxidative stress developed within the joints generate free radicles to impart lipid peroxidation, chronic inflammation, and ultimately end in the phenomenon of joint degeneration and destruction [3]. In healthy individuals, free radical production and lipid peroxidation is very low and it is balanced by the antioxidant system working in the body. When an imbalance occurs between the pro and antioxidant system, inflammatory events progress and become uncontrollable that finally result to disease state. Therefore, it's critical to supply our bodies with nutrient-dense foods, high in vitamins and antioxidants for maintaining our health.

Millets, a family of nutrient-rich, environment friendly grains, is cultivated all over the world as a crop and as fodder. They are rich in vitamins, minerals, fatty acids, phytochemicals and antioxidants which prevent health deterioration in humans such as blood pressure, cardiovascular diseases, cancer, diabetes, tumor cases etc. [4,5] Owing to many health advantages, millets are becoming more and more popular. 2018 was celebrated as National millet year and 2023 is celebrating as International millet year for their promotion among the public. Since the millets have nutraceutical properties, the current study intends to determine whether finger millet (*Eleusine coracana*), kodo millet (*Paspalum scrobiculatum*), and foxtail millet (*Setaria italica*) can withstand the antioxidant imbalance that develop in adjuvant-induced arthritic (AIA) rat models by assessing the status of their antioxidant potential.

MATERIALS AND METHODS

The study plan was approved by animal ethics committee, Committee for the purpose of control and Supervision of Experiments on Animals (CPCSEA) and strictly followed the guidelines. Female Wistar rats aged between 150±10g was divided into 6 groups with 3 rats in each group and adjuvant-induced arthritis was induced by a single intradermal injection of 0.1 ml of complete Freund's adjuvant (CFA) in hind paw. The millets used for the study were purchased from ICAR, Hyderabad. The millets steamed, ground and mixed with normal rat chow, feeding followed from 5-21 day and the grouping was as follows:

Group I- Control
Group II- AIA
Group III- AIA+10% cooked finger millet (FM)
Group IV- AIA+10% cooked kodo millet (KM)
Group V- AIA+10% cooked foxtail millet (FoM)
Group VI- AIA + Indomethacin (IND) (3mg/kg/day)

After the experimental period, the animals were sacrificed and tissues (Heart, Liver and Spleen) were collected for the analysis of antioxidant potential.

Biochemical Assays

The analysis of malondialdehyde (MDA), a lipid peroxidation product was done by TCA-TBA-HCl reagent method described by Okhawa et al. 1979 [6]. The absorbance was measured at 530 nm and concentration of MDA was expressed as mmol/g tissue. Superoxide dismutase (SOD) activity assayed by the method of Kakkar et al (1984) [7]. Absorbance was measured at 560nm. One Unit of enzyme activity is expressed as Units/mg protein. Glutathione (GSH) was estimated by the method of Benke et al, 1974 [8]. Protein estimation was done by the method of Lowry O.H. et al. 1951 using alkaline copper reagent. The color produced was measured at 560 nm.

Statistical Analysis

The results were analyzed using GraphPad Prism 5 software and statistically analyzed by one way ANOVA followed by Tukey's multiple-range tests. Data were expressed as mean ± standard error of the mean (SEM) values. P value <0.05 was considered as significant.

RESULTS

Effect of Millets on MDA Level

The rats subjected to CFA injection showed increased MDA levels in heart, liver and spleen that measured in terms of thiobarbituric acid reactive substance (TBARS). Increased MDA concentration shows increased lipid peroxidation and after millet supplementation TBARS were decreased significantly.

Table 1. Effect Of Millets On TBARS

MDA (mmols/g tissue)			
Groups	**Heart**	**Liver**	**Spleen**
I	4.21±0.83	4.73±0.74	5.17±0.91
II	16.59±1.1	19.01±0.84	17.44±0.63
III	7.9±0.75	8.1±0.64	9.3±0.59

IV	4.6±0.49	4.99±0.39	5.9±0.72
V	7.3±0.93	7.8±0.37	8.5±0.88
VI	4.31±0.98	4.94±0.31	5.21±0.58

Group I-Control, Group II- AIA, Group III- AIA+ 10% FM, Group IV- AIA+ 10% KM, Group V- AIA+ 10% FoM, Group VI- AIA+ IND. Data are expressed as Mean ± SEM values (n=3, p <0.05)

Effect of Millets on SOD Activity

The antioxidant potential of millets by SOD activity in heart, liver, and spleen are given in **Figure 2**. Adjuvant induction markedly decreased the SOD activity and after millet supplementation the activity was significantly increased as compared to normal rats.

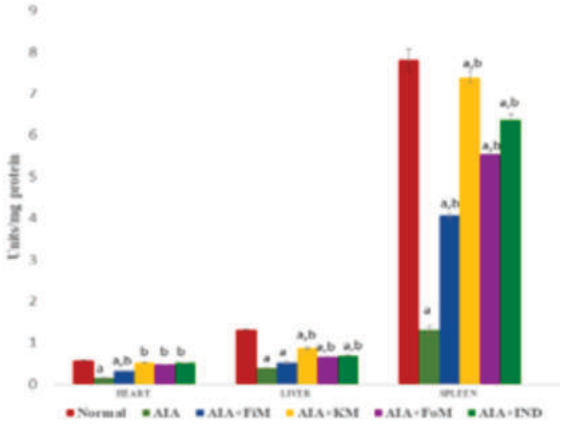

Figure 2. Effect of Millets on SOD activity Group I-Control, Group II- AIA, Group III- AIA+ 10% FM, Group IV- AIA+ 10% KM, Group V- AIA+ 10% FoM, Group VI- AIA+ IND. Data are expressed as Mean ± SEM values (n=3, p <0.05) *U- Enzyme concentration required to inhibit chromogen production by 50% in 1min.

Effect of Millets on GSH

Concentration of GSH was estimated in different tissues and a significant reduction was observed in AIA rats compared to normal rats. After millet supplementation for 21days the antioxidant status was significantly increased and IND treated group also showed similar effect.

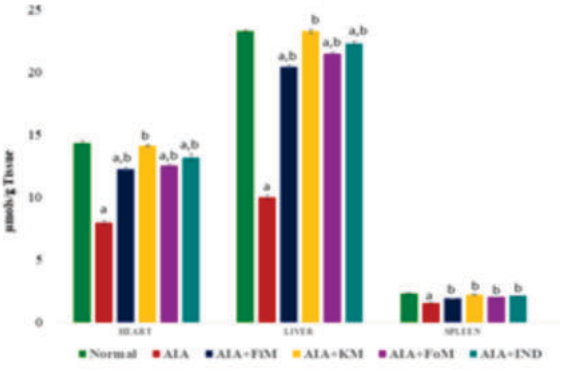

Figure 3. Effect of Millets on GSH level. Group I-Control, Group II- AIA, Group III- AIA+ 10% FM, Group IV- AIA+ 10% KM, Group V- AIA+ 10% FoM, Group VI- AIA+ IND. Data are expressed as Mean ± SEM values (n=3, p <0.05)

DISCUSSION

In this study we demonstrated the antioxidant potential of millets in rat models and it showed that 10% supplementation of Finger, Kodo, and Foxtail millets to arthritic rats can reduce the lipid peroxidation and antioxidant imbalance occurred during arthritic progression.

Millets are nutritious, gluten free cereals, rich in bioactive compounds mainly including phenolic compounds and flavonoids that are capable of managing various metabolic disorders. A number of *in vivo* studies demonstrated the beneficial effect of different millets in diabetes, cardiovascular diseases, cataract, dermal wound healing process etc. that could be due to the additive and synergistic effect of various compounds [10,11].

In this study MDA concentration was analyzed as an indicator of oxidative stress and this lipid peroxidation product increase in serum and tissues during various diseases. The MDA levels in heart, liver and spleen tissues significantly elevated in AIA rats. After 21 days of experimental period the millet supplemented groups showed decreased MDA concentration. Various studies suggest the reduction of MDA production after antioxidant supplementation [12]. During chronic inflammation important antioxidants in the body such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase etc. shows reduced activities [13]. Hence, we investigated the levels of non-enzymatic GSH and enzymatic SOD activities in model rats and millet feeding enhanced the enzyme status in different groups. It illustrates how the system's enhanced antioxidant activity effectively shielded the organism from oxidative damage by reducing the synthesis of MDA. From this it is evident that how antioxidant supplementation can improve pathological states and overall health. Chronic metabolic diseases impose enormous social and economic burdens and underutilized millets emphasize their potential as a Nutri cereal to improve the life style and prevention of non-communicable diseases. i.e., One can live a better life by including millet in the diet.

CONCLUSION

The results of our study revealed that 10% millet supplementation in normal rat feed significantly reduced the lipid peroxidation product MDA and increased the activity of antioxidant enzymes SOD and GSH. The preliminary experiment supports the antioxidant potential of finger millet, kodo millet and foxtail millet and emphasize their potential as a Nutri cereal to prevent disease progression.

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