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# Beneficial Effect of *Proso Millet* in Reducing Oxidative Stress and Osteoporosis in Post-Menopausal Women

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## Abstract

Finding out how Proso millet aqueous extract affected osteoporosis in postmenopausal women in the Guemar El-Oued area was the aim of this study. Three groups of 32 volunteers' women were chosen; as control and menopausal women have osteoporosis before and after *Proso millet* aqueous extract treatment during 30 days. Some biochemical and hematological parameters were measured. According to our findings, the osteoporosis patient group had significantly higher levels of WBC, lymphocytes, RBC, HCT, PLT, blood glucose, and serum PAL than the control group, and their serum calcium levels significantly decreased ($P<0.01$). Additionally, the findings show that, in comparison to the control group, the osteoporosis patients' group had considerably lower levels of GSH, catalase, SOD, and ORAC and significantly greater levels of MDA and vitamin C. Menopausal women who receive treatment with Proso millet aqueous extract have improved biochemical, hematological, and oxidative stress markers as well as a decreased risk of osteoporosis. In conclusion, postmenopausal women with osteoporosis benefit from phytotherapy based on Proso millet in terms of calcium status and oxidative stress.

**Keywords:** Osteoporosis, Post-menopause, Oxidative stress, *Proso millet*

## Introduction

The definitive stop of menstruating and the end of one's ability to reproduce are known as menopause. A consequence of ovarian and hypothalamic-pituitary-ovarian axis dysfunction brought on by aging (Weiss *et al*., 2004). Additionally, a major global public health problem is osteoporosis, a skeletal disorder marked by weakened bones and a higher chance of injury (Genant *et al*., 1999). Around 200 million women worldwide suffer from osteoporosis. Even while North America and Europe now have the greatest rates of osteoporosis, as population lifespan increases, emerging nations are predicted to see an increase in this risk (Sözen *et al*., 2017). One important cause of abnormalities in human metabolism and physiology is oxidative stress, as well as a number of illnesses, according to numerous recent scientific research (Atoussi *et al*., 2021). The primary cause of a number of diseases (Chetehouna *et al*., 2020) has been identified as oxidative stress, an aberrant state brought on by an excess of oxidants produced in comparison to antioxidants (Chetehouna *et al*., 2024). Numerous investigations conducted on animals and in vitro have demonstrated that oxidative stress reduces bone formation by decreasing osteoblast survival and differentiation. However, ROS also stimulate osteoclasts, which improve bone resorption (Domazetovic *et al*., 2017). Clinical research has also indicated that the pathophysiology of bone loss may include ROS and/or antioxidant systems (Abdollahi, 2005; Chidambaranathan & Culathur, 2022; Patatou *et al*., 2022; You *et al*., 2023; Pavlova, 2024). Given these findings, our research aims to investigate how millet extract protects volunteer menopausal women against osteoporosis.

## Materials and Methods

*Patients and Study Design*

This study involved 32 women between the ages of 42 and 55 who were split into two groups: 16 healthy control women with an average age of 49.234±0.34 years, 16 menopausal women with osteoporosis with an average age of 49.972±0.112 years, and the third group, which was determined by supplementing the osteoporosis group with powdered Proso millet grains for four weeks. Women with osteoporosis between the ages of 45 and 55 who voluntarily reside in the Guemar El-Oued region are included in this study, as are about control women who are healthy and free of pathologies. Additionally, all women who use medicines during menopause or who suffer from other acute or chronic diseases are not included in this study (Aloufi *et al*., 2022; Heimes *et al*., 2022). The El Oued University Ethics Committee's Department of Cellular and Molecular Biology gave its permission to the research protocol (approval number: 25 EC/DCMB/FNSL/EU2020).

*Methods and Laboratory Investigations*

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Blood is drawn from both groups after a morning fast. Following blood collection, the blood is taken in two different kinds of tubes: One is for assessing oxidative stress and hematological parameters (MDA, GSH, SOD, and CAT), while the other is for anticoagulant (EDTA) tubes. For the purpose of achieving the dosage of the following biochemical parameters: glucose, calcium, iron, PAL, vitamin C, and total antioxidant ORAC, samples are centrifuged in dry tubes for 10 minutes at 3000 rpm. The serum is then recovered.

*Analytical Methods*

Proso millet grain phytochemical screening uses established procedures to determine the phytochemical substances that are present in the grain (Dhanasekar *et al*., 2022; Saravanakumar *et al*., 2022; Ekpo *et al*., 2023; Eteng *et al*., 2023). The Slinkard and Singleton method (Boulaares *et al*., 2024) was used to quantitatively analyze the total phenols in phenolic extracts. The total quantity of flavonoid has been identified using the method outlined by Ahn *et al*. (2007). The Semi-auto Analyzer Mindray BA-88A was used to analyze serum glucose, calcium, iron, and PAL. Biomaghreb commercial kits were used for the measurements. The hematology auto analyzer (Mindray) was used to do hematological analysis (FNS).

*Preparation of Erythrocyte and Leukocyte Samples*

After centrifuging the blood EDTA tubes for 10 minutes at 2000 rpm, the plasma is removed. After 30 minutes in the freezer, the EDTA tube cap was dissolved in 50 milliliters of TBS buffer (EDTA 2.92M; tris 1.21M; pH=7). To remove the erythrocyte homogenate, the fluid was centrifuged for 10 minutes at 2500 rpm following incubation. Following erythrocyte separation, the leftover material in the EDTA tube is cleaned and spun for 30 minutes at 2,500 rpm. Until the leukocytes are homogenized and isolated, we repeat the procedure multiple times (Miller *et al*., 1988).

*Oxidative Stress Analyses*

MDA was quantified using the TBA reagent in compliance with the protocol described by Sastre *et al*. (2000). According to Weak and Cory, the quantity of reduced glutathione is determined by calculating the optical density that results from the reduction of dithio-bis-2-nitrobenzoic acid, also referred to as the Ellman reagent, with SH groups found in GSH (Weak & Cory, 1988). Employing a UV/visible spectrophotometer to measure the absorption of $H2O_2$ at 560 nm, one may calculate the catalase-triggered loss of $H_2O_2$ in the sample in line with the Aebi technique (Aebi, 1984). The spectrophotometric absorption at 560 nm, which is determined utilizing the NBT by the superoxide anion ($O_2$•) test method of SOD activity, serves as the basis for identifying the existence of SOD (Beauchamp & Fridovich, 1971). Plasma vitamin C is tested using the Jagota and Dani technique (Jagota & Dani, 1982) employing different ascorbic acids and the Folin reagent. The overall antioxidant capacity of the serum, or its ORAC (Oxygen Radical Absorbance Capacity), is determined using the Oyaizu technique, which measures the red blood cells' ability to resist hemolysis caused by free radicals in vitro with plasma.

*Statistical Analysis*

The Minitab 17 statistical assessment tool was used to compare the group's research involves employing the Student's t-test; and Microsoft Excel 2007 assisted us in creating the tests and histograms. A difference is considered statistically significant if P is less than 0.05.

## Results and Discussion

*Description of the Study Population*

Age, height, weight, body mass index, and blood type are among the general socioeconomic statistics for the two subject groups. According to **Table 1**, there are no statistically significant differences between these indicators at $P > 0.05$.

**Table 1.** Socioeconomic description of control and osteoporosis patients.

| Parameter | | Control | Patients | *P-value* |
|---|---|---|---|---|
| **Age (ys)** | | 49.234±0.341 | 49.972±0.112 | 0.114 |
| **Body Weight (kg)** | | 66.596±0.243 | 70.390±0.500 | 0.080 |
| **Height (cm)** | | 160.76±0.164 | 160.38±0.130 | 0.204 |
| **Body Mass index** | | 26.01±0.182 | 27.39±0.108 | 0.152 |
| **Blood Group (%)** | A | 43.33 | 20 | 0.04 |
| | B | 10 | 10 | 0.123 |
| | AB | 6.67 | 13 | 0.002 |
| | O | 40 | 57 | 0.032 |

*Qualitative and Quantitative Phytochemical Analysis of Proso Millet*

According to the findings of phytochemical investigations, the aqueous extract of proso millet is rich in several significant chemical components, including phenolic compounds and flavonoids in high concentrations, as well as reducing sugars, terpenoids, alkaloids, tannins, flavonoids, and saponins **(Table 2)**.

**Table 2.** Phytochemical essays, Total phenols and flavonoids concentration in *Proso millet* aqueous extract.

| Phytochemical | *P. millet* |
|---|---|
| **Flavonoids** | + |
| **Tannins** | + |
| **Alkaloids** | + |
| **Terpenoids** | + |
| **Saponins** | + |
| **Reducing compounds** | + |
| **Polyphenols (mg of GAE/g of Powder)** | 7.61±0.54 |
| **Flavonoids (mg QE/g of Powder)** | 0.70±0.01 |

*Biochemical and Hematological Parameters*

Regarding biochemical markers, **Table 3** shows that the osteoporosis patients group had considerably lower serum calcium levels (P<0.01) and significantly greater blood glucose and serum PAL activity (P<0.01) than the control group. However, compared to the osteoporosis patients group, the osteoporosis following plant extract therapy group had substantially lower blood glucose levels and serum PAL activity (P<0.001) and increased serum calcium (P0.001>). But there was no discernible change in the serum iron readings.

In the other hand, WBC (P<0.001), lymphocytes (P<0.05), red blood cells (RBC) (P<0.05), HCT (P<0.01), and PLT (P<0.05) were significantly lower in the osteoporosis+PM group than in the control group (Arios-Caro *et al*., 2022; Rudayni *et al*., 2022). Additionally, **Table 3's** results for the hematological parameters indicate that the group of osteoporosis patients had a notable rise (P<0.05) in WBC, lymphocytes, RBC, HCT, and PLT levels, but no discernible change in hemoglobin levels.

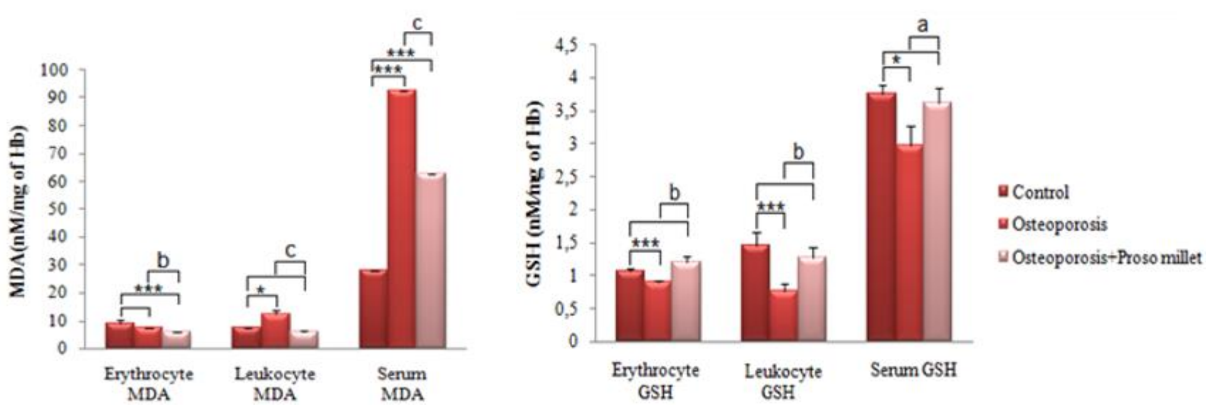
**Table 3.** Hematological levels of control and experimental group.

| Parameter | Control (n=16) | Osteoporosis (n=16) | Osteoporosis + PM (n=16) |
|---|---|---|---|
| **Blood glucose (g/l)** | 0.81±0.021 | 0.918±0.028** | 0.855±0.023 |
| **Serum Calcium (g/l)** | 74.31±1.08 | 65.15±2.04** | 75.07±1.95[c] |
| **Serum Iron (g/l)** | 14.56±1.54 | 14.94±1.48 | 14.25±1.11 |
| **Serum PAL (U/l)** | 128.8±11.0 | 183±14.0** | 107.3±15.9***[c] |
| **White Blood Cells ($\times10^3/\mu l$)** | 5.669±0.286 | 7.169±0.56* | 6.033±0.217[c] |
| **Lymphocytes ($\times10^3/\mu l$)** | 2.042±0.073 | 2.200±0.195* | 1.944±0.120[a] |
| **Hemoglobin (g/dl)** | 12.57±0.25 | 13.06±0.18 | 13.60±0.18***[b] |
| **Red Blood Cells ($\times10^6/\mu l$)** | 4.562±0.055 | 4.752±0.086* | 4.518±0.079[a] |
| **Hematocrite (%)** | 39.8±0.719 | 43.256±0.603*** | 41.262±0.498*[b] |
| **Platelets ($\times10^3/\mu l$)** | 136.06±1.86 | 255.6±16.4*** | 237.9±13.0***[a] |

*Oxidative Stress Parameters*

*Lipid Peroxidation and Reduced Glutathione Levels*

The findings in **Figure 1** demonstrate that, in comparison to the control group, the osteoporosis patients' group had considerably greater levels of MDA in their leukocytes (P<0.05) and serum (P<0.001) and considerably lower levels of GSH in their erythrocytes (P<0.001), leukocytes (P<0.001), and serum (P<0.001). The aqueous extract of Proso millet therapy resulted in a substantial drop (P<0.001) in MDA levels in leukocytes and serum and a substantial boost (P<0.01) in GSH levels in erythrocytes, leukocytes, and serum in comparison to the osteoporosis group (P<0.05).

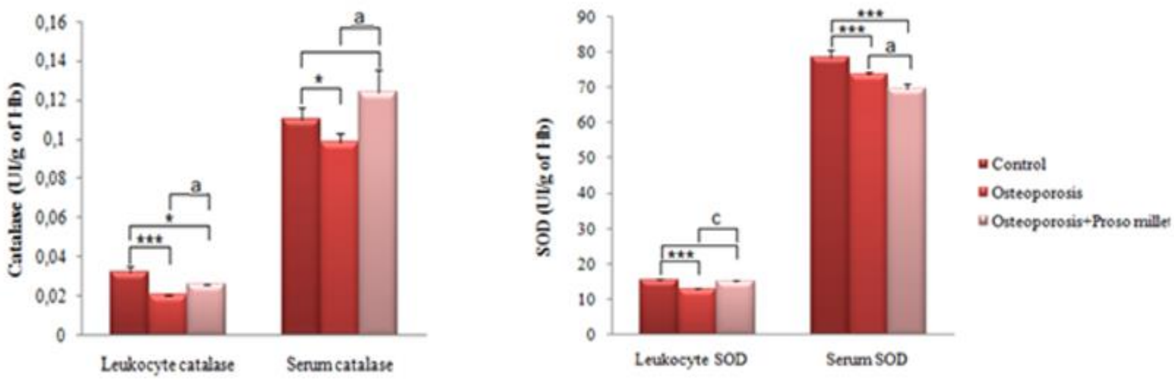



a) b)

**Figure 1.** MDA and GSH level of control and experimental group.

*Catalase and SOD Activities*

The results of one study showed that the osteoporosis patients' group had significantly lower levels of SOD and catalase activities in their serum (P<0.001, P<0.05) and leukocytes (P<0.001) than the control group **(Figure 2)**. On the other hand, the osteoporosis group after plant extract therapy showed noticeably higher levels of catalase and SOD activity in leukocytes (P<0.05, P<0.001) and in serum (P<0.05) than the osteoporosis group.

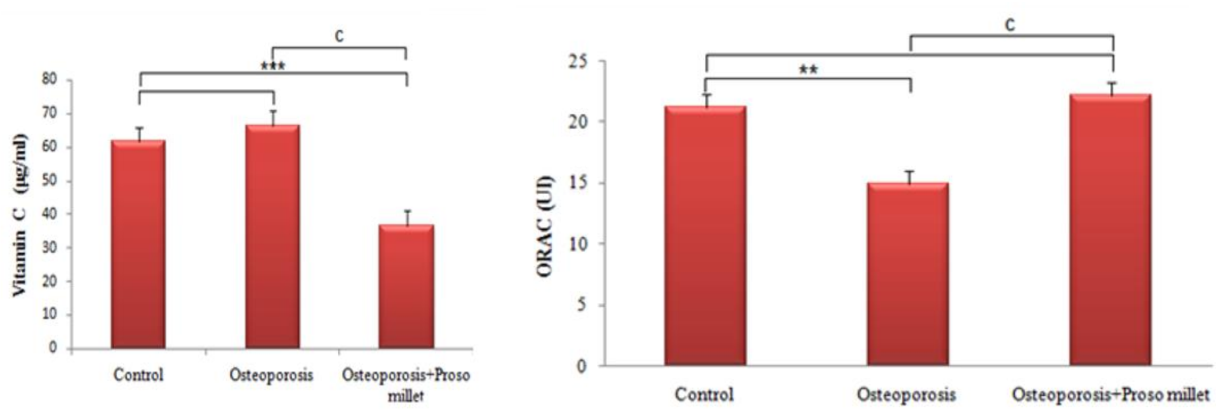



a) b)

**Figure 2.** Catalase and SOD activities of control and experimental group.

*ORAC and Vitamin C Levels*

Following the administration of an aqueous extract of proso millet, the osteoporosis patients' group's serum Oxygen Radical Absorbance Capacity (ORAC) level was considerably greater (P<0.001) than the control group, despite the fact that the ORAC level was considerably lower (P<0.01) than the control group. Additionally, there was not a statistically noteworthy variance in blood vitamin C levels between the osteoporosis group and the control group (P>0.05) as shown in the **Figure 3**.




a) b)

**Figure 3.** Vitamin C and ORAC levels of control and experimental group.

Phytochemical examination of the aqueous extract of proso millet reveals several significant secondary metabolites, such as reducing sugars, flavonoids, tannins, alkaloids, terpenoids, and saponins. Contains substantial levels of total polyphenols and flavonoids. Among other biological actions, these macromolecules exhibit antioxidant, anti-aging, anti-inflammatory, anti-diabetic, and anti-cancer properties (Beibalaeva *et al*., 2022; Osipchuk *et al*., 2022; Boulaares *et al*., 2024). Millets' phenols have been discovered to possess a variety of pharmaco-biological properties, such as anti-mutagenic, anti-oxidant, anti-viral, anti-inflammatory, and aggregation of platelets inhibitory effect (Kumar *et al*., 2018). Data showed that compared to the control, the patient group's calcium levels significantly decreased and their blood glucose and PAL activity significantly increased, which is consistent with the findings of Tirtha *et al*. who found that the postmenopausal group had a higher PAL level and a lower serum calcium level (Tirtha *et al*., 2014). When PAL was raised, all bone mineral density (BMD) readings dramatically dropped. Conversely, bone alkaline phosphatase is a skeletal health indicator that measures bone metabolism (Hailing *et al*., 2018). The physiology of humans depends on calcium. Is an essential mineral component that contributes to the rigidity of the mature bone's collagen network. One of the main factors contributing to osteoporosis and fracture is inadequate calcium accumulation, which results in a suboptimal bone mass peak and insufficient bone mineralization (DeLucia *et al*., 2003). Vitamin D may play a role in the establishment of diabetes type II mellitus, according to earlier research. In pancreatic β cells, calcium is transported across the cell membrane and calbindin, a vitamin D-dependent Ca-binding protein, is produced and regulated., are two processes that vitamin D may indirectly influence in relation to insulin release (Liefde *et al*., 2005). According to the results of our experimental study, patients with osteoporosis who had Proso millet therapy had significantly higher calcium levels than the patient group (Alharbi *et al*., 2022; Mei & Jiang, 2022; Samaranayake *et al*., 2024; Menhadji *et al*., 2024). Although millet, which includes Proso (Panicum miliaceum), gives individuals in many developing countries a lot of calories and protein (Luis *et al*., 1981), its calcium and phosphorus content is on par with other grains (Burton *et al*., 1972). According to a study on the mineral content of puffed grains (mg/100 g) (Piłat *et al*., 2016), Proso millet contains 14.75 mg/100 g of calcium, which could help raise calcium levels. Hematological parameter data indicated that the group of patients with osteoporosis had significantly higher levels of WBC, RBC, HCT, and PLT than the control group. These results provide credence to a potential connection between hematopoiesis and bone metabolism. The development of immature blood cells is known as hematopoiesis. Thus, in the aged, osteoblast activity appears to be directly and intimately linked to bone metabolism and hematopoiesis (Kim *et al*., 2011; Paspaliaris & Kolios, 2019). Niacin, riboflavin, folic acid, and hydroxycinnamic acid derivatives like p-coumaric and ferulic acid are examples of phenolic compounds that may be responsible for the osteoporosis group's significant decrease in WBC after plant therapy (Derouiche *et al*., 2022). Si *et al*. (2014) have shown that niacin lowers vascular inflammatory in both in vitro and in vivo tests by inhibiting the nuclear factor kappa B (NF-κB) signaling pathway. P-coumaric acid and hydroxycinnamic acid have also demonstrated anti-inflammatory qualities (Taofiq *et al*., 2017). As per the findings of the oxidative stress research, the blood MDA level of the osteoporosis patients was substantially greater than that of the women in the control group (İlhan *et al*., 2022; Liu *et al*., 2022; Mobeen & Dawood, 2022; Ghati *et al*., 2023). According to a study by Altindag *et al*., oxidative stress may have contributed to the altered bone metabolism in postmenopausal osteoporosis by increasing the formation of ROS in superoxide forms, as seen by elevated blood MDA levels (Chetehouna *et al*., 2024). The oxidative stress study's findings demonstrated that the sick group's levels of GSH, catalase, and substantially reduced SOD compared to the control group. A non-enzymatic antioxidant, glutathione (GSH) supports the body's defenses against oxidative stress brought on by free radicals (Chetehouna *et al*., 2024). Antioxidants play a key role in preventing postmenopausal osteoporosis, according to several studies (Maggio *et al*., 2003). When osteoporosis patients were compared to the therapy group, the aqueous extract of P. millet's influence on variables related to oxidative stress revealed an extremely substantial rise in leukocyte SOD, serum ORAC, and GSH, as well as an extremely substantial reduction in erythrocyte, leukocyte, and serum MDA levels. Because P. millet extract has anti-inflammatory and antioxidant qualities, it can lessen the effects of oxidation (Habiyaremye *et al*., 2017). Proso millet contains high concentrations of flavonoids, total polyphenols, hydroxybenzoic acid and its derivatives (vanillic acid, p-hydroxybenzoic acid, and protocatechuic acid), and hydroxycinnamic acid and its derivatives (p-coumaric acid, trans-ferulic acid, cis-ferulic acid, and 5,5′-di ferulic acid) (Chetehouna *et al*., 2024). During antioxidant processes, proso millet showed a variety of free radical scavenging properties because its phenolic and flavonoid contents predominantly serve as hydrogen donors, reducing free radical, singlet-oxygen quencher's agents, and metal chelating (Kim *et al*., 2010). The significant decrease in blood vitamin C for osteoporosis + P. millet in comparison to the osteoporosis patient group may be due to insufficient amounts of ascorbat in matured millets (Himanshu *et al*., 2018). Multiple epidemiological investigations in postmenopausal women have demonstrated inconsistencies in vitamin C consumption and bone mineral density (Boulaares *et al*., 2024). Dietary vitamin C and BMD did not independently correlate, according to the Women's Health Initiative Research (Wolf *et al*., 2005).

## Conclusion

This study discovered that the menopause stage linked to osteoporosis is marked by oxidative stress and changes in hematological and biochemical markers, which increases the likelihood of disease complications in women. However, using the plant significantly improves patients' health and lessens disease-related discomfort and problems.

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