Acta Neurophysiologica
ISSN: 2996-7554

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Research Article *Volume 6 Issue 1*

In Vivo Study of the Anti-Osteoporosis Activity of a *Polygonatum Sibiricum* Leaves against Glucocorticoid Induced Osteoporosis

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Received Date: December 26, 2024; **Published Date:** February 10, 2025

Abstract

The ethanol extract of *Polygonatum sibiricum* leaves was investigated for its anti-osteoporosis effects in a glucocorticoid-induced osteoporosis rat model. Biochemical markers such as serum calcium, phosphate, magnesium, creatinine, and alkaline phosphatase were measured. At a dose of 400 mg/kg body weight, the extract reduced serum calcium and phosphate levels by 54.01% and 27.73%, respectively, while increasing serum magnesium levels by 20% compared to the glucocorticoid-treated group. Additionally, creatinine and alkaline phosphatase levels were significantly reduced by 29.41% and 27.83%, respectively. Biomechanical analysis further supported these findings, showing that the extract at the same dose enhanced bone strength and trabecular thickness without affecting bone length compared to the diseased group. The extract also improved the cortical and trabecular bone architecture, resulting in a well-organized bone matrix. These results suggest that *Polygonatum sibiricum* leaf extract holds potential therapeutic value for treating osteoporosis.

Keywords: Anti-osteoporosis; *Polygonatum Sibiricum*; Glucocorticoid-Induced Osteoporosis; Biochemical Parameters

Abbreviations

ALP: Alkaline Phosphatase; pNPP: P-Nitrophenyl Phosphate.

Introduction

Osteoporosis, often referred to as a "silent disorder," is characterized by reduced bone mineral density and disrupted microarchitecture, leading to an increased risk of fractures with minimal or no trauma [1]. It is a systemic bone metabolic disorder typically marked by weakened bone tissue structure, thinner sclerotic layers, and heightened bone fragility [2]. The condition is primarily attributed to enhanced bone resorption, diminished bone formation, and impaired bone mineralization. Common manifestations of osteoporosis include vertebral compression fractures, distal radial fractures (Colles' fracture), kyphosis, and hip fractures. Major fracture sites include the vertebral column, hip, distal radius, and proximal femur [3].

The prevalence of osteoporosis is significantly higher in women than in men [4]. Several factors contribute to its

onset, such as genetic predisposition, an unhealthy lifestyle, aging, postmenopausal hormonal changes, inadequate physical activity, and poor dietary habits [5]. Additionally, medical conditions like hyperthyroidism, anorexia, hyperlipidemia, kidney disease, ovarian removal surgery, excessive alcohol consumption, and reduced levels of estrogen or androgen hormones are recognized as major contributors to osteoporosis development [6]. Long-term use of certain medications, including anti-epileptics, anti-cancer drugs, proton pump inhibitors, selective serotonin reuptake inhibitors, and glucocorticoids, can also induce osteoporotic conditions.

Various treatments, including antiresorptive and anabolic therapies, are employed to manage osteoporosis. Antiresorptive therapies, such as bisphosphonates, estrogen replacement therapy, and selective estrogen receptor modulators, work by reducing bone turnover and inhibiting osteoclast-mediated bone resorption [7,8].

Polygonatum sibiricum (*P. sibiricum*), commonly known as Huang Jing, is a perennial herbaceous plant belonging to the Liliaceae family [9]. In Korea, it is widely consumed as a tea. Different species of Polygonatum, such as *Polygonatum sibiricum* Gray and *P. sibiricum* Redouté, have demonstrated hypoglycemic properties in prior studies. This plant is known to lower blood glucose and lipid levels, regulate the immune system, and exhibit anti-aging effects. Despite these known benefits, there is limited research on the antioxidant and anti-inflammatory properties of water extracts from fresh *P. sibiricum*. Historically referred to as the "immortal surplus grain" and "mipu," it served as a staple food during times of famine in the Eastern Jin Dynasty due to its starch-rich roots.

The plant's leaves, flowers, fruits, and seedlings are also edible and possess pharmacological properties, including antitumor, immunomodulatory, anti-inflammatory, antibacterial, antiviral, hypoglycemic, lipid-lowering, and anti-osteoporotic effects [11-17].

This study aimed to explore the anti-osteoporotic effects of ethanol extracts from *Polygonatum sibiricum* leaves in a glucocorticoid-induced osteoporosis rat model for the first time. The research focused on the plant's flavonoid-rich composition, particularly apigenin, which plays a key role in osteoblast differentiation, bone formation, and resorption processes under oxidative stress-induced osteoporosis.

Materials and Methods

Experimental Animals

Male Wistar rats, weighing 200–220 g and aged 8–12 weeks, were selected for the study. The animals were housed in polycarbonate cages (dimensions: 430 mm × 290 mm × 180 mm) with corn cob bedding. They were maintained under controlled conditions: a temperature of 22 ± 3 °C, relative humidity of 30–70%, at least 10 air changes per hour, sound levels of 30–60 dB, and a 12-hour light/dark cycle. A standard pellet diet and water were provided ad libitum, except during fasting periods. The animals' weights were recorded before dosing. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/AOT/02/2024).

Preparation of the Plant Extract

Polygonatum sibiricum (Liliaceae), widely distributed in arid regions, has a long history of use in traditional Chinese medicine and as a functional food in China. The plant powder was procured and subjected to extraction through cold maceration using 90% v/v ethanol (3 × 1000 mL) with occasional shaking at room temperature over seven days. The extract was initially filtered using Whatman No. 1 filter paper and subsequently passed through a 0.45 µm nylon membrane. The ethanol extract was then concentrated using a rotary vacuum evaporator with a water bath maintained at 80–90 °C. The concentrated extract was stored in a desiccator at room temperature for further studies.

It rich in flavonoids, saponins, polysaccharides, a variety of amino acids needed by the human body, and other important biologically-active compounds. However, the main bioactive constituents in *P. sibiricum* are flavonoid compounds.

Flavonoids are natural compounds, secondary metabolites of plants, and the most important pigments in plants. They have antioxidant, anti-inflammatory, antibacterial, antiradiation, anticancer, antiviral, hypotensive, and other biological activities. Thus, it is highly desirable to be able to extract and make use of the abundant quantities of flavonoids in *P. sibiricum*.

Experimental Administration

A glucocorticoid-induced osteoporotic rat model was employed, using prednisolone as the representative glucocorticoid. Osteoporosis was induced by administering prednisolone orally at a dose of 28 mg/kg body weight (b.wt) daily for 25 days via oral gavage. The activity and potency of the tested samples were compared to alendronate, a standard bisphosphonate commonly used to treat glucocorticoid-induced osteoporosis. Alendronate was administered orally at a dose of 2 mg/kg b.wt daily for 25 days.

The doses of the plant extract were determined as 1/5th and 1/10th of the toxic dose, previously reported to be 2000 mg/kg b.wt by Karbalaei-doust, et al. Accordingly, the test groups received doses of 200 and 400 mg/kg b.wt of the dried Polygonatum ethanol extract, which was prepared as an oral suspension and administered orally for 25 days.

The animals were randomly divided into four groups, each consisting of six rats. The first group (normal control) received a standard diet and water ad libitum, without glucocorticoids or plant extract. The second group (disease control) was subcutaneously injected with 28 mg/kg b.wt of prednisolone daily for 25 days. The third group (standard group) received alendronate orally at 2 mg/kg b.wt daily along with subcutaneous administration of 28 mg/kg b.wt prednisolone for 25 days. The fourth and fifth groups were administered the plant extract at doses of 200 and 400 mg/kg b.wt, respectively, alongside subcutaneous prednisolone at 28 mg/kg b.wt for 25 days. Oral gavage using a feeding tube fitted with a syringe was used for all oral administrations.

On the 25th day, the animals' body weights were recorded, and samples were aseptically collected for evaluating the anti-osteoporotic effects through biochemical and biomechanical assessments. Blood samples were collected from the retro-orbital plexus under anesthesia induced by intraperitoneal administration of thiopentone sodium (23 mg/kg b.wt) using a fine capillary tube. The blood was centrifuged at 3000 rpm for 15 minutes to separate serum from cells, and the serum was stored at −20°C for further analysis.

Evaluation of the Bio-Markers, Bone Parameters and Anti-Inflammatory Markers

The serum levels of calcium, inorganic phosphorus, and magnesium were measured spectrophotometrically. Serum calcium was quantified using a colorimetric end-point method, where calcium reacts with o-cresolphthalein at pH 10–12 to form a purple complex with a maximum absorbance at 575 nm. The intensity of the color is directly proportional to the calcium concentration. A calcium colorimetric assay kit (MAK 022-1KT; Sigma Aldrich, Steinheim, Germany) was used for this analysis.

Inorganic phosphate was measured by its reaction with ammonium molybdate at acidic pH, forming a blue-colored ammonium phosphomolybdate complex with a maximum absorbance at 570 nm. This was quantified using a phosphate kit with the molybdate UV method (Cliniquant-FSR, Meril Diagnostics, New Delhi, India).

Magnesium levels were determined through its reaction with the metallochromic dye calmagite, forming a pink calmagite-magnesium complex with an absorbance maximum at 520 nm. A magnesium colorimetric assay kit (E-BC-K162-5; Elabscience, Hubei, China) was employed for this purpose. Serum alkaline phosphatase (ALP) activity was assessed by its ability to hydrolyze p-nitrophenyl phosphate (pNPP) into p-nitrophenol, a yellow product with strong absorbance at 405 nm. The rate of increase in absorbance is directly proportional to ALP activity. This was measured using an alkaline phosphatase assay kit (MAK447; Sigma Aldrich, Steinheim, Germany).

Creatinine concentration was determined through a coupled enzyme reaction, producing a colorimetric product with an absorbance maximum at 570 nm. The analysis was performed using a creatinine assay kit (MAK080; Sigma Aldrich, Steinheim, Germany). Biomechanical analysis of the femur bone was conducted to evaluate bone strength. After euthanizing the animals with an overdose of thiopental sodium, the femur bones were carefully dissected. The hip ball-and-socket joint was exposed by incision and retraction, and the bones were dislocated, cleaned of surrounding tissues, and stored in formalin. The femurs were then analyzed for breaking strength, length, weight, and thickness.

Determination of Bone Hardness

Bone hardness was assessed to evaluate the mechanical properties of the bone, which reflect the bioactivity of the tested extract. Hardness was determined by measuring the fracture point, which represents the weight at which the bone breaks. In this study, a Monsanto hardness tester was used to measure bone-breaking strength, expressed in units of kg/cm^2.

Determination of Length, Weight, and Thickness of Femur

The length was measured using a ruler, whereas the thickness was measured at the epiphyseal growth plate region using a vernier caliper. The weight of each bone was measured using digital balance.

Statistical Analysis

All the values are expressed as Mean ± SD. The data were statistically analyzed by one-way ANOVA, followed by Dunnett's test. One-way ANOVA was used to correlate the statistical difference between the variables $P < 0.05$ is considered significant.

Results

Effect of *Polygonatum sibiricum* on Serum Calcium Level in Glucocorticoid Induced Osteoporotic Rat Model

The serum calcium level serves as an indicator of bone resorption activity, with elevated levels signifying osteoporotic conditions. In this study, administration of the glucocorticoid drug prednisolone led to a substantial increase in serum calcium levels, rising by 110.77% compared to the normal control group.

Conversely, oral administration of the tested extracts significantly mitigated the osteoporotic condition, as evidenced by reductions in serum calcium levels by 45.26% and 54.01% in animals treated with 200 mg/kg and 400 mg/kg b.wt, respectively. These outcomes were comparable to those observed with alendronate, the standard reference drug, which reduced serum calcium levels by 50.36% relative to the glucocorticoid-treated group (Table 1). These findings highlight the significant impact of the tested extracts on bone resorption activity, with the most pronounced effect observed at the 400 mg/kg b.wt dose.

Effect of *Polygonatum sibiricum* on Serum Phosphate Level in Glucocorticoid Induced Osteoporotic Rat Model

Phosphate is a key mineral that crystallizes with calcium to form hydroxyapatite in bones. During bone resorption, mediated by parathyroid hormone, serum phosphate levels rise, indicating the progression of osteoporosis.

In this study, treatment with prednisolone resulted in a significant increase in serum phosphate levels, rising by 94.04% compared to the normal control group, reflecting active bone resorption and impaired bone formation. In contrast, oral administration of 200 and 400 mg/kg b.wt of *Polygonatum sibiricum* leaf ethanol extract demonstrated notable anti-osteoporotic effects, as evidenced by a decrease in serum phosphate levels by 12.63% and 27.73%, respectively, compared to the steroid-treated group. Alendronate, the standard drug, led to a 35.58% reduction in serum phosphate levels relative to the steroid-treated rats. These results suggest that the tested extracts exhibited promising anti-osteoporotic effects, promoting phosphate deposition and supporting healthy bone formation, although their impact was slightly less pronounced than that of alendronate (Table 1).

Effect of *Polygonatum sibiricum* on Serum Magnesium Level in glucocorticoid Induced Osteoporotic Rat Model

Magnesium, a trace element, plays a crucial role in hydroxyapatite crystal formation and the conversion of vitamin D to its active form. A decrease in serum magnesium levels indicates the onset of osteoporosis, as magnesium is vital for bone formation.

The results presented in Table 1 show that prednisolone administration reduced serum magnesium levels by 20.83%. In contrast, the alendronate-treated group demonstrated a 5.26% increase in serum magnesium compared to the normal control group. The diseased control group exhibited lower magnesium levels due to the formation of free radicals and impaired magnesium absorption from the diet, both of which were induced by the steroid.

However, significant improvement was observed in animals treated with 200 and 400 mg/kg b.wt of *Polygonatum sibiricum* leaf ethanol extract, leading to a 14.47% and 20.00% increase in serum magnesium levels, respectively, restoring them to normal levels.

S.No.	Treatment	Calcium mg/l	Phosphate mg/dL	Magnesium mg/dL
1	Normal Control	6.5±0.164317	4.2±0.116905	1± 0.075277
2	Disease Control (28 mg/kg b.wt of prednisolone)	14.0±0.05164	8.0±0.05164	0.8± 0.089443
3	Standard Control (alendronate 2 mg/kg b.wt + 28 mg/kg b.wt of prednisolone)	6.9±0.054772	4.9±0.104881	0.8 ± 0.10328
4	Treatment Group - EEPS 200 mg/kg + 28 mg/kg b.wt of prednisolone	7.5±0.075277	7.0±0.164317	0.9± 0.141421
5	Treatment Group - EEPS 400 mg/kg + 28 mg/kg b.wt of prednisolone	6.6±0.141421	6.1±0.121106	1±0.147196

Table 1: Effects of *Polygonatum sibiricum* on serum calcium, phosphate and magnesium levels of glucocorticoid induced osteoporosis rat model. Values are expressed as Mean ±SD (n = 6), where *p < 0.05 as compared to diseased control.

Effect of *Polygonatum sibiricum* on Serum Creatinine Level in glucocorticoid Induced Osteoporotic Rat Model

Creatinine functions as a hydroxyl radical scavenger, so higher creatinine levels are associated with increased bone resorption. According to the results in Table 2, the disease control group treated with the steroid exhibited a significant increase in serum creatinine levels, rising by 96.15% compared to the normal control group. In contrast, the standard treatment group showed a 40.20% reduction in serum creatinine levels compared to the steroid-treated group. Additionally, the groups treated with the test drug at doses of 200 and 400 mg/kg b.wt demonstrated significant

reductions in serum creatinine levels, with decreases of 15.69% and 29.41%, respectively, compared to the disease control group.

Effect of *Polygonatum sibiricum* on Serum Al alendronate, Alkaline Phosphatase Level in glucocorticoid -Induced Osteoporotic Rat Model

Serum alkaline phosphatase levels serve as an indicator of increased bone activity, with higher levels suggesting heightened bone turnover and potential future bone loss. The results presented in Table 2 show that the diseased control group had a significant increase in serum alkaline phosphatase levels, rising by 79.02% compared to the normal control group. In contrast, the alendronate-treated group exhibited a 44.74% reduction in alkaline phosphatase levels compared to the diseased group.

Additionally, the groups treated with the sample at doses of 200 and 400 mg/kg b.wt showed significant reductions in alkaline phosphatase levels, with decreases of 20.41% and 27.83%, respectively, compared to the diseased control group. The ethanol extract of *Polygonatum sibiricum* leaves significantly lowered alkaline phosphatase levels in a dose-dependent manner, with the 400 mg/kg b.wt dose showing results similar to those of the standard and normal control groups.

S.No.	Treatment	Serum Creatinine mg/dL	Serum alkaline phosphate µg/ml
1	Normal Control	0.52±0.012111	104±1.67332
2	Disease Control (28 mg/kg b.wt of prednisolone)	1.12±0.015166	180±1.632993
3	Standard Control (alendronate 2 mg/kg b.wt + 28 mg/kg b.wt of prednisolone)	0.89±0.028752	101±2.160247
4	Treatment Group - EEPS 200 mg/kg + 28 mg/kg b.wt of prednisolone	0.77±0.016021	144±1.21106
5	Treatment Group - EEPS 400 mg/kg + 28 mg/kg b.wt of prednisolone	0.62±0.023381	135±1.414214

Table 2: Effect of *polygonatum sibiricum* Leaves total ethanol extract on serum creatinine (mg/dL) and alkaline phosphatase level (µg/ml). Values are expressed as Mean ±SD (n = 6). Significantly different from the diseased group at *p < 0.05.

Determination of the Bone strength, thickness and length Parameters

The mechanical properties of the bone were evaluated by assessing cortical and trabecular strength as well as bone mineralization. The fracture point was determined by applying weight to the femur bone. The results revealed that the diseased group treated with prednisolone exhibited increased bone porosity and fractured with less weight compared to the normal control group. In contrast, the ethanol extract of *Polygonatum sibiricum* leaves, administered at a dose of 400 mg/kg b.wt, demonstrated enhanced bone strength compared to the diseased animals, with results approaching those of the standard and normal control groups. Notably, while there were no significant differences in bone length between the groups, the test drug group exhibited greater bone thickness than the disease control group (Table 3).

S.NO.	Treatment	Bone breaking strength	Bone thickness (mm)	Bone length (cm)
1	Normal Control	4.3±0.231661	5.51±0.076594	3.5±0.216025
2	Disease Control (28 mg/kg b.wt of prednisolone)	3.6±0.225832	3.3±0.257559	3.5±0.233809
3	Standard Control (alendronate 2 mg/kg b.wt + 28 mg/kg b.wt of prednisolone)	3.8±0.240139	4.7±0.126491	3.5±0.207364
4	Treatment Group - EEPS 200 mg/kg + 28 mg/kg b.wt of prednisolone	3.6±0.187083	3.87±0.314113	3.6±0.228035
5	Treatment Group - EEPS 400 mg/kg + 28 mg/kg b.wt of prednisolone	4.2±0.303315	4.80±0.303315	4.2±0.303315

Table 3: Effect of *polygonatum sibiricum* leaves ethanol extract on bone strength, thickness and length parameters. Values are expressed as mean ± SD (n = 6). Significantly different from the diseased group * p < 0.05.

Discussion

The anti-osteoporotic effects of the ethanol extract from *Polygonatum sibiricum* leaves were investigated for the first time using a steroid-induced osteoporotic rat model. This study was designed based on the plant's rich flavonoid content, particularly apigenin, which is known to play a key role in osteoblast differentiation, growth, and bone formation and resorption processes in oxidative stress-induced osteoporosis.

The primary aim was to determine if the flavonoid and phenolic compounds in *Polygonatum sibiricum* leaves could prevent and protect against bone damage caused by corticosteroid-induced oxidative stress. The treatment was administered prophylactically over a 28-day period, with the ethanol extract given orally at doses of 200 and 400 mg/kg, while prednisolone was administered at a dose of 28 mg/kg starting on day 28. While steroids are beneficial in treating severe conditions such as organ transplantation, asthma, arthritis, and pneumonia, prolonged use can lead to significant bone resorption, resulting in osteoporosis. This condition often remains asymptomatic until bone fractures occur [18-22]. Chamomile flowers, another plant with medicinal properties, contain various bioactive compounds, including alpha-bisabolol, sesquiterpenes, coumarins such as herniarin and umbelliferone, phenylpropanoids like chlorogenic and caffeic acids, and flavones like apigenin, luteolin, quercetin, and rutin, which contribute to the plant's therapeutic effects.

Alkaline phosphatase is an enzyme essential for bone matrix mineralization and plays a role in osteoblast differentiation, bio-mineral tissue development, cementum mineralization, skeletal development, and responses to various compounds such as vitamin D, antibiotics, glucocorticoids, and cyclic organic compounds. Elevated serum alkaline phosphatase levels can indicate several conditions, including celiac disease, biliary obstruction, liver disease, leukemia, lymphoma, sarcoidosis, and myocardial infarction. It is also commonly elevated in bone disorders such as Paget's disease, osteomalacia, osteoblastic bone tumors, and osteoporosis. In this study, the ethanol extract of *Polygonatum sibiricum* leaves significantly regulated alkaline phosphatase levels in serum samples from treated rats [23].

Serum calcium and phosphate concentrations are crucial indicators of bone activity. These ions bind to bone tissue to form hydroxyapatite crystals, which are essential for storing calcium and phosphate and maintaining bone strength. Elevated serum calcium and phosphate levels often indicate osteoporosis. Magnesium, a trace element, plays a vital role in bone mineralization and resorption, with 60% of the body's magnesium stored in hydroxyapatite crystals. Magnesium is also important for converting vitamin D into its active form, enhancing calcium and phosphate absorption. Magnesium deficiency can contribute to osteoporosis by disrupting crystal formation and promoting osteoclast activity. The results presented in Table 1 show that serum calcium and phosphate levels significantly increased, while serum magnesium levels decreased in animals treated with prednisolone, indicating oxidative stress and bone resorption. However, treatment with *Polygonatum sibiricum* leaf extracts significantly decreased calcium and phosphate levels and increased serum magnesium levels, suggesting that the extract mitigated steroid-induced bone resorption [24].

Serum creatinine levels are an important marker for bone health, as elevated levels are associated with decreased bone mineral density and increased bone resorption. Creatinine acts as a hydroxyl radical scavenger, reducing oxidative stress and regulating bone resorption. In steroid-induced oxidative stress, pro-inflammatory mediators such as cytokines and interleukins promote osteoclast differentiation and increase free radical production, which accelerates bone resorption. The high phenolic content in *Polygonatum sibiricum* leaves is crucial in scavenging free radicals, reducing oxidative stress, and preventing bone resorption, ultimately aiding in bone mineralization [25].

The osteoprotective effects of *Polygonatum sibiricum* extract were also demonstrated in biomechanical parameters, such as bone breaking strength, thickness, and length. Table 3 shows significant improvements in these measures in the extract-treated groups compared to the diseased control group. The diseased control group, which received prednisolone, exhibited lower bone breaking strength due to increased trabecular porosity. In contrast, the plant extract-treated groups showed marked improvements in bone strength, approaching the levels seen in both the normal and standard control groups. This highlights the negative impact of steroids on bone health, disrupting the balance between bone resorption and formation, thereby increasing fracture risk. Steroids inhibit collagen synthesis, hinder osteoblast differentiation, induce osteoblast apoptosis, and accelerate osteoclast maturation, all of which contribute to osteoporosis. *Polygonatum sibiricum* leaves are rich in secondary metabolites, particularly flavonoids, sesquiterpenes, coumarins, and glycosides, which effectively prevent bone damage caused by oxidative stress-mediated osteoporosis.

The flavonoids in the plant, including apigenin, kaempferol, and luteolin, have shown significant potential in reducing oxidative stress and preventing bone loss. Apigenin, in particular, is an effective free radical scavenger that combats oxidative damage induced by steroids. It also plays a crucial

role in osteoblast differentiation and growth. As a potent antioxidant, apigenin helps maintain mineral concentration and plasma antioxidant levels while mediating key cell signaling pathways involved in bone formation [26,27].

Conclusion

In conclusion, the study provides compelling evidence that the ethanol extract of *Polygonatum sibiricum* leaves possesses strong anti-osteoporotic effects in a steroid-induced osteoporotic rat model. This activity is primarily attributed to the plant's abundant bioactive flavonoids, particularly apigenin, which plays a crucial role in osteoblast differentiation, bone formation, and protection against oxidative stress-induced bone resorption. The prophylactic administration of the plant extract at doses of 200 and 400 mg/kg for 28 days effectively countered the detrimental effects of prednisolone on bone health. Steroid-induced osteoporosis, a serious consequence of prolonged corticosteroid use, disrupts the balance between bone resorption and formation, elevates fracture risk, and often remains asymptomatic until significant bone damage occurs.

The study demonstrated that the *Polygonatum sibiricum* extract significantly reduced serum calcium and phosphate levels, markers of bone resorption, while increasing serum magnesium levels, an indicator of bone mineralization. These results suggest that the extract mitigates the oxidative stress and bone degradation caused by corticosteroids. Additionally, the extract's ability to regulate key bone markers such as alkaline phosphatase and creatinine further emphasizes its potential in improving bone metabolism and preventing osteoporosis. The phenolic compounds in the extract, especially flavonoids like apigenin, kaempferol, and luteolin, possess potent antioxidant properties that help scavenge free radicals, reduce lipid peroxidation, and prevent osteoclast-mediated bone resorption.

Moreover, the observed improvements in biomechanical parameters, including bone strength, thickness, and length, further support the extract's protective effects on bone health. Overall, the findings suggest that *Polygonatum sibiricum* leaves could serve as a valuable natural therapeutic for preventing and managing steroid-induced osteoporosis, providing a safer alternative to conventional treatments.

Acknowledgments

We acknowledge the significant advice and support provided by the faculty of Pharmacology at Seven Hills College of Pharmacy, Tirupati and Toxgene AR Biolabs Private Limited, Narasinga Puram, Andhra Pradesh, in completing this study within the given time frame.

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