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RESEARCH ARTICLE

Ameliorative effects of microalgal *Spirulina* protein fortified with leaf powder of *Moringa* and finger millet on glucocorticoid induced osteoporosis in rats

Vijay Kumar, V.T. Shilpa*, B.C. Girish, G.B. Manjunatha Reddy[1], S.P. Satheesha[2], N.M. Rajashailesha[3], K.R. Anjan Kumar, P. Ravikumar[4] and S.N. Pramod[5]

Department of Veterinary Pathology, Veterinary College, Hassan, KVAFSU, Bidar, [1]NIVEDI-ICAR, Bengaluru, India, [2]Department of Veterinary Public Health and Epidemiology, [3]Department of Veterinary Anatomy, [4]Department of Veterinary Pathology, Veterinary College, Shivamogga, India, [5]Department of Studies in Biochemistry and Food Technology, Davangere University, Shivagangotri, Davanagere, India

Address for Correspondence

V.T. Shilpa, Assistant Professor, Department of Veterinary Pathology, Veterinary College, Hassan, KVAFSU, Bidar, India, E-mail: drshilpavt@gmail.com

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ABSTRACT

The current investigation was conducted to assess the anti-osteoporotic potential of microalgal *Spirulina* protein fortified with leaf powder of *Moringa* and Finger millet (MSMF) in female Wistar rat model of Methylprednisolone induced osteoporosis. The study included six groups of six rats each. Group I (normal control) rats, received distilled water orally daily for seven weeks, Group II (disease control) to VI rats received Methylprednisolone @ 40 mg/kg bw subcutaneously thrice a week for six weeks from 2nd to 7th week of the experimental period. Additionally, Group III (reference control) rats received Alendronate at 40 µg/kg body weight subcutaneously three times a week for the same duration. Group IV, V and VI rats received MSMF @ 500, 1000 and 2000 mg/kg bw respectively, daily orally for seven weeks, along with Methylprednisolone. Group II rats showed significant decrease in feed intake, body weight, femur weight, haemato-biochemical parameters, radiological and histopathological parameters of the femur. The changes caused by Methylprednisolone were alleviated in Alendronate group and MSMF treatment groups in a dose dependent manner. Histopathologically, the MSMF treatment groups showed substantial improvement in trabecular thickness, cortex thickness, osteocyte number, reduction in resorption cavities and erosion of cartilage. The anti-osteoporotic efficacy of MSMF @ 500 mg/kg was lower than that of Alendronate, while the MSMF @ 1000 mg/kg and 2000 mg/kg bw showed better efficacy than MSMF @ 500 mg/kg and equivalent to the effects of Alendronate.

Keywords: Finger millet, methylprednisolone, microalgal *Spirulina* protein, *moringa*, Osteoporosis

INTRODUCTION

Osteoporosis is a systemic condition associated with aging that typically affects mammals, primarily humans. It is a condition marked by reduced bone density and the weakening of bone structure, which results in increased fragility and vulnerability to fractures[1]. About one in three women and one in five men over 50 years will suffer a fracture due to osteoporosis[2]. These numbers emphasize the importance of awareness, early detection and proactive measures to prevent osteoporotic fractures.

Osteoporosis arises through either primary or secondary causes. Primary osteoporosis is due to rapid bone loss following menopause due to oestrogen decline and gradual age-related bone loss in older individuals, affecting both men and women[3]. Secondary osteoporosis arises due to many causes including hypogonadism, endocrine disorders, gastrointestinal diseases, transplantation, genetic disorders and drugs such as anticoagulants, glucocorticoids, anti-convulsants etc. Among drug-induced osteoporosis cases, glucocorticoids are the most frequent cause. Glucocorticoids, directly and indirectly impact the skeleton, mainly affecting osteoblasts and osteocytes. They promote the formation of osteoclasts, leading to increased bone breakdown[4].

Glucocorticoids are widely used as the standard treatment for alleviating inflammation and to promote immunosuppression in conditions such as asthma, allergic reactions, rheumatoid diseases, dermatological issues, collagen and vascular disorders, inflammatory bowel disease, other systemic illnesses and ocular inflammatory diseases. However, use of glucocorticoids with higher doses and for prolonged periods may increase the likelihood of osteonecrosis and osteoporosis[5].

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Table 1. The mean (± SE) body weight (g) values of rats of different experimental groups in the study at weekly interval.

Groups	Day 0	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	7th Week
Group I (NC)	227.0 ± 5.63^{A}	231.0 ± 5.63^{A}	232.7 ± 4.65^{A}	254.0 ± 3.13^{Ba}	263.5 ± 1.23^{BCa}	268.2 ± 2.41^{Ca}	271.3 ± 3.65^{Ca}	275.5 ± 3.60^{Ca}
Group II (DC)	226.8 ± 5.27^{A}	230.8 ± 5.27^{A}	229.2 ± 4.83^{A}	210.0 ± 4.13^{Bb}	208.8 ± 5.11^{Bb}	218.2 ± 3.02^{ABb}	225.0 ± 7.76^{Ab}	228.5 ± 4.11^{Ab}
Group III (RC)	227.2 ± 6.21^{A}	231.2 ± 6.21^{A}	230.8 ± 3.76^{A}	237.7 ± 3.02^{ABc}	251.0 ± 3.71^{BCa}	258.8 ± 2.84^{Ca}	262.2 ± 2.04^{Ca}	263.7 ± 4.77^{Ca}
Group IV (MSMF @ 500)	227.5 ± 6.71^{A}	231.5 ± 6.71^{A}	236.5 ± 4.58^{AB}	247.0 ± 4.85^{BCac}	253.0 ± 4.11^{CDa}	260.0 ± 3.40^{CDa}	262.7 ± 3.87^{Da}	264.5 ± 4.94^{Da}
Group V (MSMF @ 1000)	227.7 ± 7.64^{A}	231.7 ± 7.64^{A}	235.7 ± 5.39^{AB}	248.3 ± 4.59^{BCac}	257.5 ± 3.06^{CDa}	260.7 ± 2.68^{CDa}	261.5 ± 2.58^{CDa}	265.8 ± 5.57^{Da}
Group VI (MSMF @ 2000)	227.8 ± 7.20^{A}	231.8 ± 7.20^{A}	238.3 ± 4.98^{AB}	248.7 ± 5.47^{BCac}	256.5 ± 4.73^{CDa}	264.0 ± 3.44^{Da}	264.0 ± 2.74^{Da}	266.5 ± 4.97^{Da}

Mean ± SE bearing different superscripts (abcd; within column and ABCD; between columns) are statistically significant at $p < 0.05$ (n = 6)

Various drugs targeting bone resorption or formation have been developed to address bone loss, but they are often ineffective for primary and secondary osteoporosis and may cause adverse side effects. As a result, herbal remedies are used as alternatives as they are widely accepted, accessible, affordable and believed to have fewer side effects[6].

Spirulina platensis is a type of blue-green microalgae which has been recognized for its potential as a calcium source. Since, *Spirulina* is rich in protein and minerals, it has garnered attention for its ability to boost mineral absorption by influencing intestinal microflora[7]. *Moringa Oleifera* leaves are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper. Additionally, they contain abundant phytosterols such as stigmasterol, sitosterol and kampesterol, serves as precursors for oestrogen production which can have positive effect on bone[8]. Finger millet has all the quantitative and qualitative attributes needed to be a prototype for calcium bio fortification. It notably emerges as the most abundant source of calcium among all cereal grains contributing to bone strength[9].

To avoid osteopenia and osteoporosis, it's necessary to take calcium and vitamin D supplements. *Spirulina, Moringa oleifera* leaves and Finger millet have good amounts of calcium and phytochemicals which prevents bone loss. The present research work was undertaken to investigate the protective effects of microalgal *Spirulina* protein fortified with leaf powder of *Moringa* and Finger millet (MSMF) on glucocorticoid induced osteoporosis in female rats.

MATERIALS AND METHODS

Animals

The study was conducted on 36 adult female Wistar rats, of six month age, weighing around 200 to 250g, procured from Spring Labs, Vasanthanarasapura, Tumkur, Karnataka (Reg. No. 2259/PORcbiVt/S/23/CCSEA). All the rats were acclimatized to standard laboratory conditions for seven days prior initiation of the experiment and maintained at 22±3^{O}C temperature, humidity of 30 to 70% and 12-hour light/dark cycle throughout the study period. Rats provided with regular standard pellet diet along with free access to deionized drinking water *ad libitum* throughout the course of the experiment. The animals were handled as per CCSEA guidelines and study was approved by the Institutional Animal Ethical Committee (HVC/IAEC/08/2024).

Preparation and administration of drug

Methylprednisolone sodium succinate (MP) was procured from NEON Laboratories Limited, Mumbai, India. It was diluted in distilled water and injected subcutaneously at 40 mg/kg bw. The MSMF, which was extracted, purified and having composition of 55 g of brown ragi, 20 g of *Moringa* powder, 15 g of *Spirulina* and 10 g of jaggery powder per 100 g was obtained from the Department of Food Technology, Davangere University, Shivagangotri, Davangere. It was used at the dose rate of 500, 1000 and 2000 mg/kg bw orally. Alendronate was procured from Sigma Aldrich Corporation, St. Louis, USA and was injected subcutaneously at the rate of 40 µg/kg bw in distilled water.

Experimental design

The study included six treatment groups comprising of six rats in each group. Group I served as normal control (NC) and received distilled water orally throughout the experiment and subcutaneously from 2nd week to end of 7th week; Group II disease control (DC) rats

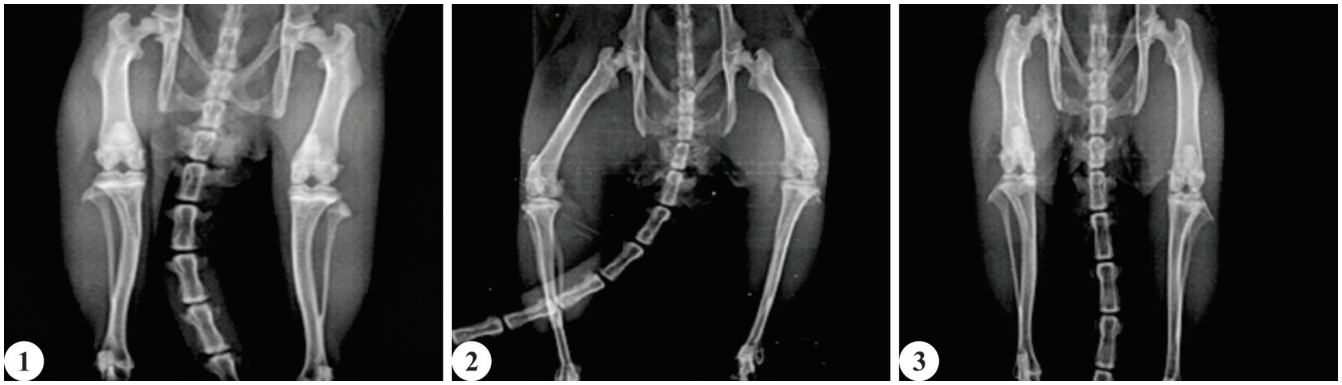

Fig. 1. Dorso-ventral radiograph of femur of control Group I rat showing normal cortex thickness and radiodensity; **Fig. 2.** Dorso-ventral radiograph of femur of disease control Group II rat showing reduction in cortex thickness and reduced radiodensity; **Fig. 3.** Dorso-ventral radiograph of femur of Group VI rat showing improved cortex thickness and radiodensity.

received MP at 40 mg/kg bw, subcutaneously, thrice a week for six weeks (from 2nd to 7th week) and distilled water orally throughout the experiment; Group III (RC) rats along with MP treatment received Alendronate subcutaneously thrice a week (from 2nd to 7th week); Group IV rats along with MP treatment received MSMF at 500 mg/kg bw daily from 1st week to 7th week; Group V rats along with MP treatment received MSMF at 1000 mg/kg bw daily from 1st week to 7th week; while Group VI rats along with MP treatment received MSMF at 2000 mg/kg bw daily from 1st week to 7th week.

Parameters studied

Body weight (g) and feed (g) consumption were assessed every week. At the end of the study (49th day), all rats were anaesthetized by using Ketamine and Xylazine (as I/M injection) to take lateral and anterio-posterior radiographs of femurs of animals. The radiographic images were acquired using 63 kVp, 8 mA and exposure time of 0.06 s with focal distance of 30 mm. Radio density of bones was used for evaluation of osteoporosis in long bones of rats. Blood was collected from retro-orbital plexus in EDTA and serum vials for hematological and biochemical analysis, respectively. All animals were sacrificed humanely and were subjected for detailed post-mortem examination. The mean femur weight in grams at the end of the study were recorded.

Histopathology

Representative femur samples from rats of all the groups were subjected to histopathological studies. The tissue was fixed using 10% Neutral Buffered Formalin solution and decalcified by formic acid (90%) sodium citrate method. Sections were prepared using paraffin blocks and stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT) after dewaxing[10].

Histopathological scoring

The femur samples were examined in random microscopic areas and the changes were assessed by counting 20 different non overlapped fields for the same slide of each animal[11]. Cortical bone thickness (µm) was measured under low power field from the periosteum to endosteum at three different points in each section; osteocyte lacunae with or without nuclei were counted in oil immersion to know the severity of osteoporosis.

Statistical analysis

Statistical analysis of the data collected for various parameters was done using one-way ANOVA with Tukey's test and two-way ANOVA with Duncan's post hoc test[12].

RESULTS

General observations

Group I rats remained active throughout the period

Table 2. The mean (±SE) values of various haematological parameters of rats in different groups on day 49th of the study.

Groups	TEC (10^6/µl)	TLC (10^3/µl)	Hb (g/dl)	Platelet (10^3/µl)	PCV (%)
Group I (NC)	5.04 ± 0.10^{a}	8.43 ± 0.12^{a}	13.02 ± 0.52^{a}	494.0 ± 18.65^{a}	40.50 ± 1.68^{a}
Group II (DC)	3.77 ± 0.15^{d}	4.48 ± 0.11^{d}	9.68 ± 0.38^{b}	299.7 ± 10.64^{b}	32.47 ± 1.06^{b}
Group III (RC)	4.02 ± 0.17^{cd}	5.43 ± 0.12^{c}	11.82 ± 0.45^{a}	360.0 ± 9.52^{b}	34.75 ± 0.76^{bc}
Group IV (MSMF @ 500)	4.16 ± 0.09^{bd}	6.93 ± 0.08^{b}	12.17 ± 0.29^{a}	435.2 ± 15.94^{a}	36.88 ± 1.64^{ab}
Group V (MSMF @ 1000)	4.56 ± 0.09^{abc}	7.12 ± 0.11^{b}	12.37 ± 0.32^{a}	463.2 ± 14.01^{a}	36.58 ± 1.19^{ab}
Group VI (MSMF @ 2000)	4.67 ± 0.16^{ab}	7.10 ± 0.08^{b}	12.73 ± 0.27^{a}	450.5 ± 14.62^{a}	38.55 ± 1.27^{ac}

Mean ± SE values with different superscript differ significantly at $p < 0.05$ (n = 6)

of experiment. Group II rats exhibited clinical signs such as reduced feed intake, dullness, reduced body weight, arched back, ruffled fur, looked restless and were difficult to handle. The rats of Group III to VI manifested similar clinical signs as that of disease control rats, but with reduced intensity and frequency.

Feed consumption

Mean weekly feed consumption in Group I rats were normal and progressive. Group II rats showed significant decrease in

Table 3. The mean (±SE) values of various serum biochemical parameters of rats in different groups on day 49th of the study.

Groups	Calcium (mg/dl)	Phosphorous (mg/dl)	ALP (IU/L)
Group I (NC)	9.93 ± 0.28[a]	4.92 ± 0.17[a]	76.33 ± 3.32[a]
Group II (DC)	8.12 ± 0.21[b]	4.07 ± 0.28[b]	44.50 ± 1.78[c]
Group III (RC)	9.37 ± 0.40[ab]	4.63 ± 0.12[ab]	75.00 ± 2.38[a]
Group IV (MSMF @ 500)	8.75 ± 0.26[ab]	4.45 ± 0.11[ab]	58.33 ± 1.87[b]
Group V (MSMF @ 1000)	9.08 ± 0.35[ab]	4.33 ± 0.18[ab]	64.67 ± 1.84[b]
Group VI (MSMF @ 2000)	9.22 ± 0.42[ab]	4.55 ± 0.19[ab]	67.17 ± 1.81[ab]

Mean values with different superscript differ significantly at $p < 0.05$ (n = 6)

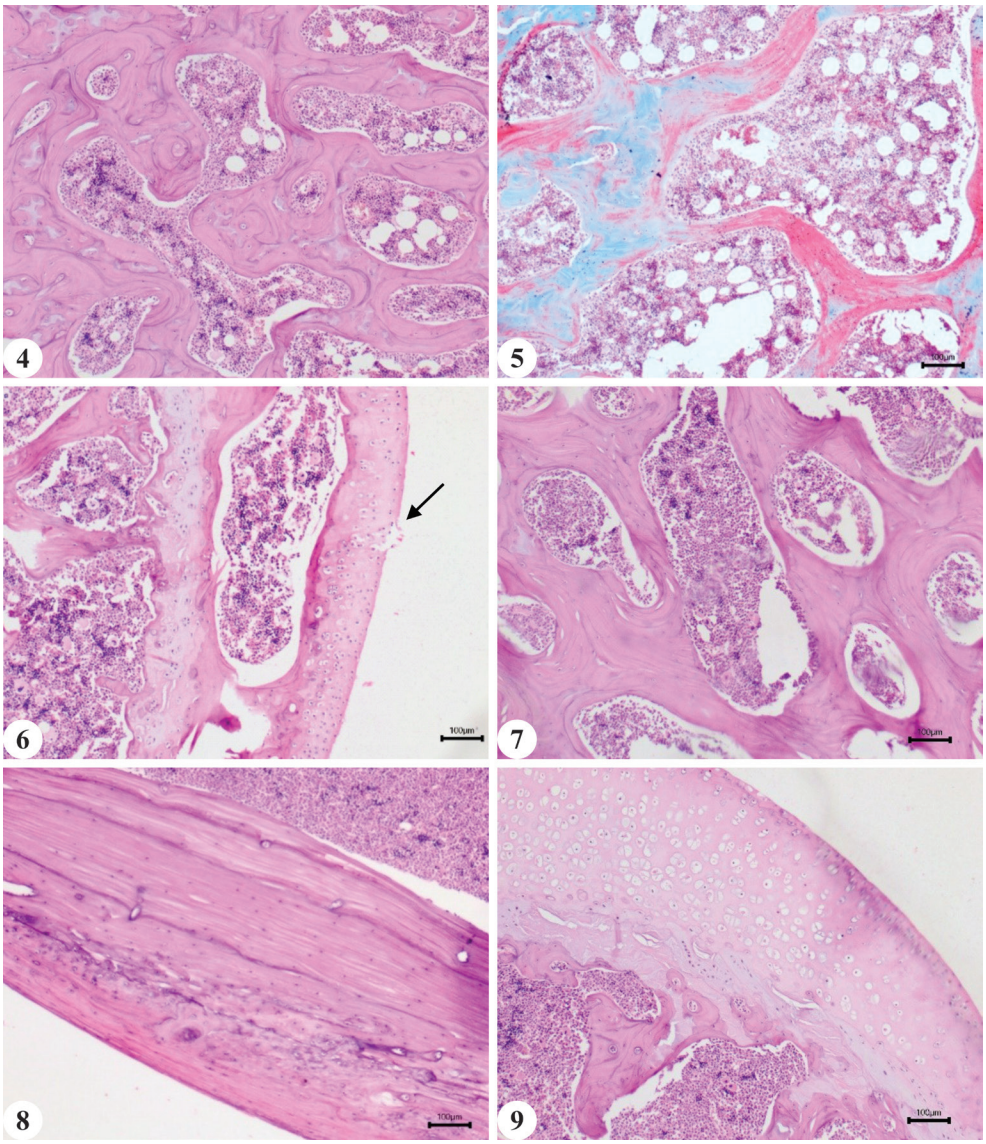

Fig. 4. Femur head from Group I rat showing normal architecture of trabecular bone with thick network of trabeculae and regular bone marrow spaces (H&E X100); **Fig. 5.** Femur bone from Group II rat showing thinning of trabeculae with reduced collagen and wider bone marrow spaces (MT X100); **Fig. 6.** Femur bone from Group II rat showing reduced cartilage thickness and with erosion (arrow) (H&E X100); **Fig. 7.** Femur head from Group VI rat showing moderate improvement in the thickness of trabeculae with increased collagen and reduced widening of bone marrow spaces (H&E X100); **Fig. 8.** Femur bone from Group IV rat showing improvement in the cortical thickness of compact bone and increased cellularity (H&E X100); **Fig. 9.** Femur head from Group VI rat showing moderate improvement in cartilage thickness with reduced break in between cartilage (H&E X100).

feed consumption of about 5.29%, 10.99%, 11.23%, 8.49%, 7.72% and 8.40% during 2nd, 3rd, 4th, 5th, 6th and 7th week, respectively as compared to Group I. During first two weeks, Group V rats showed a significantly ($p < 0.05$) higher feed intake compared to other groups. From 3rd to 7th week, all the treatment groups showed a significant increase in weekly feed consumption compared to Group II rats.

Body weight

The mean±SE body weights at different time intervals of 0 day and 1st, 2nd, 3rd, 4th, 5th, 6th and 7th week of experiment are presented in Table 1. Group I rats remained healthy throughout the period of experiment and showed progressive enhancement in their body weight over the course of experiment. Body weight of Group II rats decreased significantly from 3rd to 7th week compared to Group I. The animals of Group IV to VI showed significant improvement in the body weight from 3rd to 7th week when compared to Group II.

Radiography

Radiographic images of femur and tibia of Group II rats revealed decreased radio density (bone density) with thinning of cortical bone and increased medullary cavity. Also, diaphyseal fracture of tibia was seen in one rat in Group II. In the treatment groups (Group IV to VI), the radiographic images of femur and tibia showed no significant difference in radio density, cortex thickness and were comparable to that of control rats. Also, the treatment groups showed significant improvement in bone density and cortex thickness of femur and tibia when compared to Group II with no evidence of fractures (Fig. 1 to 3).

Organ weight

Group II showed significant ($p < 0.05$) decrease in absolute weight of femur in comparison to Group I, where as in Group III, IV, V and VI, femur weights were significantly ($p < 0.05$) higher than Group II.

Haematology parameters

The mean±SE values of hematological parameters on 49th day of the experiment are presented in Table 2. The mean values of Total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin (Hb), platelets (PLT) and packed cell volume (PCV) of Group II rats were significantly ($p < 0.05$) decreased when compared to Group I rats. The TEC in Group V and VI; the TLC, Hb and PLT in Group IV to VI rats; the PCV in Group VI were increased significantly ($p < 0.05$) as compared to Group II rats. The TEC values of Group V rats and PCV values of Group V and VI rats were slightly increased as compared to Group II rats.

Serum biochemistry

The mean±SE values on 49th day of the experiment are presented in Table 3.

Serum calcium

There was a significant ($p < 0.05$) decrease in serum calcium levels of Group II rats by 18.22% than Group I rats. Among the treatment groups, there was no significant difference in mean serum calcium levels. The mean values of serum calcium levels of Group III, IV, V and VI rats were improved than Group II rats.

Serum phosphorous

There was a significant ($p < 0.05$) decrease in serum phosphorous levels of Group II rats by 17.28% than Group I rats. Among the treatment groups, there was no significant difference in mean serum phosphorous levels. The mean values of serum phosphorous levels of Group III, IV, V and VI rats were improved than Group II rats.

Alkaline phosphatase (ALP)

There was a significant ($p < 0.05$) decrease in serum ALP levels of Group II rats by 41.70% than Group I rats. Among the treatment groups (Group IV to VI), there was no significant difference in mean serum ALP levels. The mean values of serum ALP levels of Group III, IV, V and VI rats were significantly ($p < 0.05$) improved than Group II rats.

Gross pathology

The rats from Group II showed reduction in the femur length and reduction in the size of lymph node in four out of six rats. The rats from Group III to VI did not reveal any visible gross abnormalities.

Histopathology

Table 4. The mean (±SE) values of femur cortex thickness (μm), osteocyte number and number of empty lacunae (per field in oil immersion) of rats in different groups on final day (49th day) of the study.

Groups	Femur cortex thickness	Osteocyte number	Number of empty lacunae
Group I (NC)	682.3 ± 10.89^{a}	10.50 ± 0.56^{a}	2.45 ± 0.25^{a}
Group II (DC)	461.9 ± 11.31^{b}	6.60 ± 0.41^{b}	5.30 ± 0.33^{b}
Group III (RC)	621.9 ± 9.59^{c}	10.00 ± 0.47^{a}	2.95 ± 0.28^{a}
Group IV (MSMF @ 500)	482.7 ± 12.70^{b}	9.30 ± 0.42^{a}	4.15 ± 0.23^{c}
Group V (MSMF @ 1000)	580.7 ± 8.21^{c}	9.75 ± 0.45^{a}	3.35 ± 0.23^{ac}
Group VI (MSMF @ 2000)	596.6 ± 6.75^{c}	9.85 ± 0.55^{a}	3.25 ± 0.30^{ac}

Mean values with different superscript differ significantly at $p < 0.05$ (n = 6)

The femur of the Group II rats showed decreased cortical thickness of compact bone, reduced osteocyte and osteoblast numbers, increased number of empty lacunae without osteocytes, erosion of periosteal and endosteal layers. The cortical bones showed cracks and fissures with few abnormal osteocytes and haversian canals. There was loss of normal architecture of inner cancellous bone trabeculae with thinning of trabeculae thickness, wider bone marrow spaces filled with increased number of adipocytes and discontinuous bony ossicles. Further, the cartilage thickness was reduced along with break in between or incomplete cartilage when compared to control Group I rats (Fig. 4 to 6). Group III rats revealed microscopical changes similar to that of Group II rats but with mild improvement. The femur of Group IV to VI rats showed mild to moderate improvement in the cortical thickness of compact bone, minimal to moderate increase in cellularity, decreased empty lacunae and increased mineralisation when compared to Group II rats. There was mild improvement in the endosteal surface with reduced erosion. The cracks and fissures of cortical bones were reduced. There was mild improvement in the loss of normal architecture of inner cancellous bone trabeculae with minimal improvement in trabeculae thickness and reduced bone marrow spaces. The thickness of cartilage was improved with reduced break in between when compared to Group II rats (Fig. 7 to 9).

Histopathological score of femur

The HP score of all sectioned femur samples assessed for parameters like femur cortex thickness and osteocyte lacunae with or without nuclei are presented in the Table 4. Group II rats showed significant ($p < 0.05$) decrease in mean femur cortical thickness and mean number of osteocyte lacunae with nuclei and significant ($p < 0.05$) increase in mean number of osteocyte lacunae without nuclei when compared to Group I rats. The mean femur cortex thickness was significantly ($p < 0.05$) higher in Group III, V and VI but non-significantly higher in Group IV rats in comparison to Group II rats. The mean osteocyte lacunae with nuclei was significantly ($p < 0.05$) higher in Group III to VI in comparison to Group II rats. The mean osteocyte lacunae without nuclei were significantly ($p < 0.05$) lower in Group III to VI in comparison to Group II rats.

DISCUSSION

Glucocorticoid induced reduction in femur weight were in accordance with the reports of previous findings[13,14]. MP induced decrease in haematological parameters like TEC, TLC, Hb, PLT and PCV values were similar to those previously reported[14]. Decline in blood parameters could be associated with the negative effects of glucocorticoids on bone marrow and intravascular lysis and decreased release of immune cells from the bone marrow[15].

The TEC, TLC, PLT and Hb values in all the MSMF treated groups (Groups IV to VI) were improved significantly compared to Group II rats. Studies on animal models documented that phycocyanin of *Spirulina* induces the expression of bcl-2 in haematopoietic cells that may inhibit apoptosis. There is also evidence that c-phycocyanin and polysaccharides of *Spirulina* enhance white blood cell production[16]. It plays a role in improves immune system efficiency, as well as playing an important part in stimulation of the erythropoiesis[17]. It was opined that increased haematological values in *Moringa* leaves administration was related to the components such as protein amino acids, vitamins B, E and iron[18]. Finger millet has good amounts of thiamine, riboflavin, iron, methionine, isoleucine, leucine, phenylalanine, essential amino acids and polyphenols[19] which may enhance the production of haematological parameters.

A significant decrease in the mean values of serum calcium, phosphorous and alkaline phosphatase was observed in the disease control rats[13,20]. It has been previously highlighted the significance of these biochemical markers in detecting osteoporosis resulting from Glucocorticoid administration[13,15]. Glucocorticoid usage may cause decrease in calcium and phosphorous absorption in the intestine and decreased renal reabsorption of calcium and phosphorus[21]. The reduction in ALP is due to inhibition of osteoblastogenesis and apoptotic effects of glucocorticoids on both osteoblasts and osteocytes[22].

The improved serum calcium, phosphorous and ALP levels in MSMF treated groups may be attributed due to presence of rich minerals, vitamins in *Spirulina* and improved mineral absorption in intestine by positive effect on intestinal microflora by *Spirulina*[23], due to *Moringa* leaf which are rich in flavonoids, phytoestrogens and phytochemicals like kaempferol and quercetin which has osteoblastic potential and prevents osteoclastic resorption[24].

MP administration also caused a significant reduction in radio density (bone density) of femur and tibia with thinning of cortical bone and increased medullary cavity. Further, diaphyseal fracture of tibia was seen in one out of six rats. These findings were previously reported earlier[14,21] and they opined that long term use of glucocorticoids causes bone resorption and decreased bone mineral density.

The histopathological examination of femur from rats in the disease control group revealed decreased cortical thickness of femur, reduced osteocyte and osteoblast numbers, increased number of empty lacunae without osteocytes[11]. The endosteal surface was irregular[25], cortex showed cracks and fissures with few abnormal

osteocytes and haversian canals. The intercellular substance consisted of non-homogenous ground matrix with several resorption cavities within the matrix when compared to normal control group rats. The lamellae of the compact bone were reduced in thickness[11] when compared to normal control rats. Further, there was loss of normal architecture of inner cancellous bone trabeculae with thinning of trabeculae thickness, discontinuous bony ossicles and wider bone marrow spaces filled with increased number of adipocytes[23,26]. Histological changes in disease control rats may be due to use of glucocorticoids. Glucocorticoids inhibit expression of genes important for bone formation including those responsible for the production of collagen A1 TGF-β, fibronectin and IGF-1. They, also increase production of receptor activator of NF-kB ligand (RANKL) and reduced production of osteoprotegerin, resulting in increased osteoclast recruitment and survival[4,22]. Glucocorticoids induce apoptosis in osteoblasts and osteocytes by activating GSK-3 and caspase-3[27]. They lower IGF-1 gene transcription in osteoblasts leading to decrease production of type 1 collagen[4]. When glucocorticoids cause osteocyte apoptosis, this network may be disrupted, impairing the repair process and leading to micro damage accumulation and increased bone fragility[22].

Improvement in the treatment groups (Group IV, V and VI) may be attributed to the synergistic antiosteoporotic effects of *Spirulina*, *Moringa* leaves powder and Finger millet. *Spirulina* have been shown to reduce osteoclast formation by decreasing the number of TRAcP and RANKL-positive cells[28]. *Moringa* is abundant in flavonoids and phytoestrogens, which stimulate osteoblasts by acting through the estrogen receptor signalling and by reducing the RANKL-mediated osteoclastogenesis[13]. Further, ragi is rich source of calcium may be attributed to the improved microarchitecture and bone mineral density.

The current research showed that among the treatment groups, notably, histological changes of femur in rats from Group V and VI were less severe compared to those in Group IV and Group II rats. The anti-osteoporotic effect of MSMF in the present study suggests that *Spirulina* microalgae, leaf powder of *Moringa* and Finger millet can be used in combination as a potential preventive agent or as a supplement in treatment of osteoporosis. However, the underlying mechanisms that may play a therapeutic role in the prevention and control of osteoporosis by MSMF warrant further mechanistic studies to elucidate the mechanism of action.

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