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Research Article

PRECLINICAL STUDIES OF VARIOUS EXTRACTS OF *POLYALTHIA LONGIFOLIA* FOR THE MANAGEMENT OF DEXAMETHASONE INDUCED OSTEOPOROSIS IN RATS

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ABSTRACT

Polyalthia longifolia is known to be used traditionally for the treatment of many diseases such as helminthiasis, diabetes and hypertension etc., and it is not investigated earlier for the treatment of osteoporosis. The aim of the present work is to investigate the effects of *Polyalthia longifolia* for the management of dexamethasone induced osteoporosis in rats. Female Wister rats were used to investigate antiosteoporotic activity of various extracts i.e. petroleum ether, chloroform, ethyl acetate, ethanol and water of *Polyalthia longifolia* at the dose of 100mg/kg b. wt. and 200mg/kg b.w. and observed various parameters i.e. alkaline phosphatase estimation, change in body weight, bone density, bone mechanical strength, and ash value were determined in dexamethasone induced osteoporosis. Alkaline phosphatase (ALP) level was found to be increased in osteoporotic group than normal control group. Ethyl acetate extract shows best results in bone density and percentage ash value. While aqueous extract shows best results in biomechanical strength and body weight of rats. The results obtained in the present study provide evidence that *Polyalthia longifolia* contributes to the healing of osteoporosis.

Keywords: Osteoporosis, *Polyalthia longifolia*, Dexamethasone, Alkaline phosphatase, Bone density

INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone density and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility. Loss of bone density occurs with advancing age and rates of fracture increase markedly with age, giving rise to significant morbidity and some mortality.

Osteoporosis is estimated to affect over 200 million people worldwide[1]. One in 3 women older than 50 years will eventually experience osteoporotic fractures, as will 1 in 5 men[2]. By 2050, the worldwide incidence of hip fracture is projected to increase by 240% in women and 310% in men[3]. Existing drugs used for osteoporosis are biphosphonates, raloxifene, teriparatide and oestrogens which have some side effects such as gastrointestinal disturbance, peptic ulcers, nausea, dizziness etc.; to overcome these side effects herbal drugs are being explored.

Polyalthia longifolia (sonn.) Thwaites (Annonaceae) is native to the drier regions of India and is locally known as "Ashoka" and is commonly cultivated in India, Pakistan, and Sri Lanka. *P. longifolia*, although an ornamental tree, finds its reference in Indian medicinal literature owing to its popular Hindi name Ashoka. *Polyalthia longifolia* is reported to contain, cytotoxic aporphine alkaloid such as liriodenine, noroliveroline, oliveroline-beta-N-oxide and azafluorene alkaloids like darienine, polyfothine and isooncodine, polyfothine and isooncodine [4], clerodane diterpene, 16-oxo-cleroda-3, 13(14)Z-dien-15-oic acid, which was named polyalthialdoic acid (3) [5]. *Polyalthia longifolia* is one such drug which is reported to be used traditionally for many disease [6] and is investigated to have cytotoxic, antitumor [7], antimicrobial [8], antifungal [9] and hepatoprotective activity[10] and it is not investigated earlier for its anti -osteoporotic activity.

Phytoestrogen compounds, such as quercitin, kaempferol[11] and phenolic compounds[12], were found to be effective in osteoclast resorption activity and induce mature osteoclast apoptosis *in vitro*, indicating a possible anti-resorptive activity[13]. Since, *Polyalthia longifolia* is reported to contain, phenolic compounds flavonoids, Sterols, Tannins. So, it is hypothesized that *Polyalthia longifolia* may contain anti-osteoporotic activity. Corticosteroids cause osteoporosis and fractures in a high percentage of patients. The study aims to examine and determine the effects of various extracts of *Polyalthia longifolia* for the management of dexamethasone induced osteoporosis.

MATERIAL AND METHODS

Collection, Authentication and Drying of plant material

The bark of *Polyalthia longifolia* tree was collected from Kamla Garden, Bhopal district of Madhya Pradesh, India. The selected plant for the study was identified and authenticated by Botanist Dr.Zia-ul Hasan, Safia College and a voucher specimen no. 363/bot/safia/10 was submitted. Bark was dried and powdered plant barks of *Polyalthia longifolia* was successively extracted with various solvents viz petroleum ether, chloroform, ethyl acetate, ethanol and water (40-60 ^{0}C) using soxhlet apparatus and extracts were vaccum dried.

Phytochemical screening

In preliminary phytochemical screening, various chemical tests were performed for the identification of common phytoconstituents in *Polyalthia longifolia* extracts[14-15].

Animals

A total of 54 female rats weighing between 100-150g were housed in the animal house. The rats were housed in sanitized polypropylene cages containing paddy husk as bedding. The animals were maintained under controlled conditions of temperature (23±2°C), humidity (50±5%), and a 12-h light–dark cycle. All animals were allowed free access to water and fed on a commercial diet. All the studies conducted were approved by the Institutional animal ethical committee (Registration No. 778/03/C/CPCSEA), VNS Institute of Pharmacy, Bhopal.

Acute toxicity

A safe oral dose of the extract was determined by acute toxic class method of organization of economic co-operation and development (OECD) as per 423 guidelines[16]. The extracts were found to be safe upto 2000 mg/kg body weight.

Induction of osteoporosis

After 7 days of acclimatization, experimental animals (female Wister rats) were divided into groups (Each group having six experimental animals) and osteoporosis was induced by administrating decadon (dexamethasone sodium phosphate) 7mg/kg b.wt. (Wockhardt 4mg/2ml) intramuscularly [11] once a week up to four weeks. Weights of rats were observed during induction of osteoporosis and their treatment.

Antiosteoporotic study

Antiosteoporotic activity was performed using dexamethasone induced osteoporosis in rats. Animals were divided into fourteen groups. Group I was kept as control group, group II was served as osteoporotic control, group III were given dexamethasone (once a week up to four weeks) & sodium alendronate (daily), group IV were given dexamethasone (once a week up to four weeks) & pet. ether extract (daily 100mg/kg), group V were given dexamethasone (once a week up to four weeks) & chloroform extract (daily 100mg/kg), group VI were given dexamethasone (once a week up to four weeks) & ethyl acetate extract (daily 100mg/kg), group VII were given dexamethasone (once a week up to four weeks) & ethanol extract (daily 100mg/kg) group VIII were given dexamethasone (once a week up to four weeks) & aqueous extract (daily 100mg/kg), group IX were given dexamethasone (once a week up to four weeks) & pet. ether extract (daily 200mg/kg), group X were given dexamethasone (once a week up to four weeks) & chloroform extract (daily 200mg/kg), group XI were given dexamethasone (once a week up to four weeks) & ethyl acetate extract (daily 200mg/kg), group XII were given dexamethasone (once a week up to four weeks) & ethanol extract (daily 200mg/kg), group XIII were given dexamethasone (once a week up to four weeks) & aqueous extract (daily 200mg/kg) and group XIV was kept as vehicle control.

The experimental groups *i.e.* Group I was served as control (without treatment with dexamethasone), Group II as osteoporotic control, Group III was treated with standard drug sodium alendronate, rest of the Groups {IV, V, VI, VII, VIII (100mg/kg)} & Groups {IX, X, XI, XII, XIII (200mg/kg)} were treated with different extract (petroleum ether, chloroform ,ethyl acetate, ethanol and water) of *Polyalthia longifolia* (orally at concentration of 100mg/kg and 200mg/kg) and Group 14 was served as vehicle control.

Analysis of Various parameters

Alkaline phosphatase level

Serum alkaline phosphatase level is increased in disease of bone. Transient increase elevations may be found during healing of bone fractures. Physiological bone growth also elevates ALP in serum[13]. Serum ALP level was detected for all groups.

Measurement of body weight

All rats were regularly weighed from starting till the end of protocol. Decrease in body weight was observed during long term dose of dexamethasone sodium phosphate, further, gradually increase in body weight of rats was observed during treatment with plant extracts.

Measurement of femur length- The femur length was measured as the distance between the greater trochanter and the medial condyle of the left femur using vernier calipers.

Measurement of bone density- Air entrapped in the pores of bones was removed using vacuum pump. Density of bone was calculated by weighing bones and volume of the same was measured in volumetric flask.

Femur mechanical strength- Femur mechanical strength was measured using three point bending technique. Femur was fixed at two ends and weights were suspended using string at the middle of the bone. Weight at which the bone breaks is the mechanical strength of the bone.

Ash value- Ash value is the measure of inorganic contents of bone. Femur was kept at 600°c in muffle furnace till the weight of bone become constant. Final weight of bone was measured.

Statistical analysis

The statistical significance was assessed using one way analysis of varience (ANOVA) followed by Dunnette's multiple comparision test. The values are expressed as mean ± SEM and P<0.05 was considered significance.

RESULTS AND DISCUSSION

The present study was performed to evaluate the effect of various extracts of Polyalthia longifolia for the management of dexamethasone induced osteoporosis. The powdered bark of Polyalthia longifolia was successively extracted with various solvents viz petroleum ether, chloroform, ethyl acetate ethanol and aqueous. The yields were found to be 0.68g (0.34%w/w of crude drug) of pet. ether extract with semisolid mass of yellow colour, 4.4g (2.2%w/w of crude drug) of chloroform extract with greenish brown colour semisolid mass, 10.4g (5.2%w/w of crude drug) of ethyl acetate extract with red colour semisolid mass, 20.8g (10.45w/w of crude drug) of ethanol extract with brown colour semisolid mass and 34g (17%w/w of crude drug) of aqueous extract with dark brown colour semisolid mass. The phytochemical tests confirmed the presence of common constituents such as petroleum ether extract contains fats & steroids, chloroform extract contain steroids, ethyl acetate extract contains steroids, while ethanol and aqueous extracts contains flavonoids, tannins and phenolic compounds.

Osteoporosis was induced by dexamethasone sodium phosphate 7mg/kg body wt. administered by intramuscular route (decadon-4mg/2ml). Osteoporosis treatment was done by petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of Polyalthia longifolia.

Alkaline phosphatase level

Effect of dexamethasone sodium phosphate on alkaline phosphatase level of female rats was found to be increased than the normal level (16-50u/l in 100mg rats) in blood serum of osteoporosis induced rats in compare to control rats. It infers that osteoporosis was induced in all rats other than control rats while treated rats shown higher alkaline phosphatase level than osteoporotic rats which indicates prevention of bone loss by plant extracts (Table 1 & Table 2).

Measurement of body weight

Body weight of all treated groups on 31st day was found to be significant (P<0.01) when compared to control group. The mean body weight of rats in different groups were measured and maximum increase in body weight was seen in aqueous extract i.e. 147.85±0.7195 mg as compared to osteoporotic group which is 69.55±1.115 mg (Table 3).

Measurement of femur length

The length was measured of left femur using vernier calipers but no significant result was obtained as there was no difference found in length during the experiment.

Table 1: Effect of dexamethasone sodium phosphate on alkaline phosphatase level of female rats (before treatment)

Group Dose	Control	Osteoporotic	Standard	Pet ether Extract	Chloroform Extract	Ethyl acetate extract	Ethanol Extract	Aqueous extract
100mg/kg	62.67 ±3.3	131.9 ±29.48*	405.266 ±33.67*	186.29 ±11.35*	309 ±25.00*	334.4 ±15.0*	373.86 ±35.7*	370.05 ±02*
200mg/kg	62.67 ±3.3	131.9 ±29.48*	405.266 ±33.67*	309.366 ±13.17*	281.1 ±27.00*	505.86 ±44.5*	490.6 ±5.7*	457.70 ±17.5*

n=6 albino rats per group; all values are unit per litre (u/l); Values are represents mean ± SEM, *P<0.01 (Comparison of osteoporotic and extract group with control group)

Table 2: Effect of various extracts of *Polyalthia longifolia* on dexamethasone sodium phosphate induced osteoporosis on alkaline phosphatase level in female rats (after treatment)

Group Dose	Control	Osteoporotic	Standard	Pet ether Extract	Chloroform Extract	Ethyl acetate extract	Ethanol Extract	Aqueous extract
100mg/kg	52 ±03.5	340 ±2*	568.2 ±31*	355 ±11.25*	334.26 ±21.3*	362.33 ±1.7*	473 ±15*	407.4 ±12*
200mg/kg	52 ±03.5	340 ±2*	568.2 ±31*	668.8 ±0.9*	503 ±20*	555 ±15*	553.73 ±5.86*	506.33 ±0.35*

n=6 albino rats per group; all values are in unit per litre (u/l); Values are represents mean ± SEM, *P<0.01 (Comparison of osteoporotic and extract group with control group)

Table 3: Effect of different extracts of *Polyalthia longifolia* on Body weight

Group Dose	Control	Osteoporotic	Standard	Pet. ether Extract	Chloroform Extract	Ethyl acetate extract	Ethanol Extract	Aqueous extract
100mg/kg	12.22 ±0.25	69.55 ±1.115*	125.5 ±1.37*	129.9 ±2.40*	100.69 ±2.75*	118.3 ±6.35*	102.5 ±7.8*	136.4 ±4.67*
200mg/kg	12.22 ±0.25	69.55 ±1.115*	125.5 ±1.37*	133.5 ±3.306*	112.1 ±10.0*	115.9 ±8.21*	108.33 ±5.18*	147.85 ±0.7195*

n=6 albino rats per group; all values are in unit per litre (u/l); Values are represents mean ± SEM, *P<0.01 (Comparison of osteoporotic and extract group with control group)

Measurement of bone density

Extracts treated groups shown significant increase in bone density when compared to osteoporotic groups (P< 0.01). The maximum increase in bone density was seen in ethyl acetate 200mg/kg dose i.e. 1.963±0.01 in comparison to osteoporotic group which was found to be 0.2881±0.049 (Table 4).

Femur mechanical strength

The mean biomechanical strength of femur in different groups was observed and maximum increase in biomechanical strength was observed in aqueous extract i.e. 3.45±.07731kg in comparision to osteoporotic group which was 0.164±0.17kg (Table 5).

Ash value

Percentage ash value of femur for all treated groups was found to be significant (P<0.01) than osteoporotic group. The mean Percentage ash value of femur in different groups was observed and maximum increase was observed in ethyl acetate extract i.e 40.075±.4926 mg comparison to osteoporotic group which was 13.92±4249 mg (Table 6).

Table 4: Effect of different extracts of *Polyalthia longifolia* on Bone density of femur

Group Dose	Control	Osteoporotic	Standard	Pet ether Extract	Chloroform Extract	Ethyl acetate extract	Ethanol Extract	Aqueous extract
100mg/kg	2.328 ±0.04	0.2881 ±0.049*	1.978 ±0.040*	0.526 ±0.011*	1.216 ±0.131*	1.9 ±0.01*	1.403 ±0.03*	1.055 ±0.09*
200mg/kg	2.328 ±0.04	0.2881 ±0.049*	1.978 ±0.040*	0.998 ±0.248*	1.335 ±0.074*	1.963 ±0.01*	1.92 ±0.021*	1.2425 ±0.003*

n=6 albino rats per group; all values are in cubic centimetre; Values are represents mean ± SEM, *P<0.01 (Comparison of osteoporotic, extract and control group)

Table 5: Effect of different extract of *Polyalthia longifolia* on Biomechanical strength of femur

Group Dose	Control	Osteoporotic	Standard	Pet ether Extract	Chloroform Extract	Ethyl acetate extract	Ethanol Extract	Aqueous extract
100mg/kg	4.418 ±1.32	0.164 ±0.17*	3.6333 ±0.74*	0.629 ±0.43*	1.42 ±0.32*	2.702 ±0.17*	2.619 ±0.17*	2.98 ±0.335*
200mg/kg	4.418 ±1.32	0.164 ±0.17*	3.6333 ±0.74*	1.701 ±0.769*	2.749 ±0.289*	2.98 ±0.11*	3.248 ±0.36*	3.45 ±0.77*

n=6 albino rats per group; all values are in kilogram; Values are represents mean ± SEM, *P<0.01 (Comparison of osteoporotic, extract and control group)

Table 6: Effect of different extract of *Polyalthia longifolia* percentage ash value of femur

Group Dose	Control	Osteoporotic	Standard	Pet ether Extract	Chloroform Extract	Ethyl acetate extract	Ethanol Extract	Aqueous extract
100mg/kg	47.41 ±0.81	13.92 ±0.42*	41.98 ±0.51*	24.84 ±0.43*	27.68 ±0.29*	38.33 ±0.22*	38.43 ±2.41*	29.236 ±0.342*
200mg/kg	47.41 ±0.81	13.92 ±0.42*	41.98 ±0.51*	33.45 ±1.113*	27.11 ±0.2692*	40.075 ±0.49*	35.80 ±0.64*	27.79 ±0.607*

n=6 albino rats per group; all values are in milligram; Values are represents mean ± SEM, *P<0.01 (Comparison of osteoporotic, extract and control group.

Alkaline phosphatase (ALP) is an enzyme present in many tissue cells like liver, kidney, placenta, and germ cells and in osteoblasts. Bone ALP has become the clinically most relevant enzyme in the diagnosis of bone disease. The ALP concentration is increased in osteoporotic rats than the normal rats because of osteoclasts causing the increase in bone resorption, simultaneously with the intensification of resorption process; the bone formation process is also increased by the enhancement of the osteoblast activity. It led to growth of ALP activity in the osteoporotic group [17] whereas treated group also shows increase in ALP concentration in blood than the osteoporotic group. It indicates calcification[18].

Experimental data indicates that the treated rats have shown increased alkaline phosphatase level in blood serum of rats, increased bone density, biomechanical strength and ash value in treated rats in compare to osteoporotic rats. Previous studies have suggested that glucocorticoids have a direct effects on the osteoblast cells and alter the number of osteoblasts by mechanism i.e. induction of apoptosis[19]. Presently, the best osteoporosis treatment is the biphosphonates, compounds that decrease the osteoclast resorptive activity. This decrease alters the structural formation of the osteoclast, thereby causing its cellular death. [20]

To our knowledge, the biological activity of *Polyalthia longifolia* is due to phytosteroids, flavonoids, and phenolic compounds [21-23]. It was an effort to examine all options available with which osteoporosis management may be bettered for the benefit of all by isolating and characterizing drugs from plant sources having potent antiosteoporotic activity.

CONCLUSION

There are many synthetic drugs to treat osteoporosis, but there is still loophole in the natural medicine, which may have fewer side effects. Phytoconstituents like flavonoids, glycosides and calcium are reported to contain antiosteoporotic activity. The phytochemical screening of *Polyalthia longifolia* confirmed the presence of common constituents such as lipids, sterols, glycosides, flavonoids, tannins and phenolic compounds.

In vivo studies were performed for osteoporosis management induced by dexamethasone. Alkaline phosphatase level was checked before i.e. on 0 day and after treatment i.e. on the 31st day in blood serum of rats which were observed higher than normal, which indicates increase resorption activity of osteoclasts in the osteoporotic group. Change in the alkaline phosphatase level in the blood serum was found to be significantly ($p<0.5$) increased. Bone density of the extract group was observed to be more than the osteoporotic group but not from the standard group.

The result obtained in the present study provided evidence that *Polyalthia longifolia* contributes to the healing of osteoporosis.

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