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PHARMACOLOGICAL EVALUATION FOR ANTI-OSTEOPOROTIC ACTIVITY OF HERBAL EXTRACT

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ABSTRACT

Osteoporosis is a progressive metabolic bone disorder characterized by decreased bone mass and microarchitectural deterioration, leading to increased fracture risk. Current pharmacological treatments are effective but often associated with adverse effects, prompting the search for safer, natural alternatives. This study aimed to evaluate the anti-osteoporotic potential of the ethanolic extract of *Curcuma caesia* rhizome in a dexamethasone-induced osteoporotic rat model. *Curcuma caesia* rhizomes were extracted using ethanol, yielding 8.25% w/w extract. Phytochemical screening revealed the presence of alkaloids, flavonoids, diterpenes, phenols, proteins, and carbohydrates. Osteoporosis was induced using dexamethasone, and treatment effects were assessed on serum calcium and phosphorus levels, as well as femur weight, length, and bone calcium content. Raloxifene (5.4 mg/kg) was used as a standard drug. The extract at doses of 100 mg/kg and 200 mg/kg significantly improved serum calcium and phosphorus levels and restored femoral parameters compared to the osteoporotic control group. The 200 mg/kg dose showed effects comparable to raloxifene, indicating a dose-dependent protective effect. The phytochemicals present, especially flavonoids and phenols, may contribute to the observed bone-conserving effects through antioxidant and anti-inflammatory mechanisms. The ethanolic extract of *Curcuma caesia* rhizome exhibits significant anti-osteoporotic activity in dexamethasone-induced rats, supporting its potential as a natural therapeutic agent for osteoporosis management. Further studies are needed to elucidate its molecular mechanisms and clinical relevance.

Keywords: *Curcuma caesia*, Anti-osteoporotic, Dexamethasone-induced osteoporosis, Phytochemicals, Bone mineral content, Herbal medicine.

INTRODUCTION

Osteoporosis is a chronic skeletal disorder characterized by decreased bone mass and deterioration of bone microarchitecture, resulting in increased bone fragility and susceptibility to fractures (Kanis *et al.*, 2008). The condition predominantly affects postmenopausal women due to estrogen deficiency, which accelerates bone resorption and impairs bone formation (Compston *et al.*, 2019). Although current therapeutic agents such as bisphosphonates, calcitonin, and hormone replacement therapy offer clinical benefits, they are often limited by adverse effects, long-term safety concerns, and high treatment costs (Johnell & Kanis, 2006). These limitations have led to a growing interest in identifying safer, natural alternatives for the prevention and treatment of osteoporosis.

Medicinal plants have long been explored for their bone-protective effects due to their multi-targeted actions, safety profile, and affordability. Among them, *Curcuma caesia* Roxb. commonly known as black turmeric is a lesser-known but traditionally significant rhizomatous herb belonging to the Zingiberaceae family. It has been used in Ayurvedic and folk medicine for various ailments, including inflammation, pain, and bone-related disorders (Gupta *et al.,* 2012). Phytochemical studies have shown that *Curcuma caesia* contains curcuminoids, essential oils (such as camphor and ar-turmerone), flavonoids, and other polyphenols known for their antioxidant, anti-inflammatory, and estrogenic properties (Mishra *et al.,* 2011; Singh *et al.,* 2013).

Oxidative stress and inflammation play a critical role in the pathogenesis of osteoporosis by promoting osteoclastogenesis and impairing osteoblast function (Manolagas, 2010). The rich antioxidant and anti-inflammatory phytoconstituents of *Curcuma caesia* suggest its potential to modulate bone remodeling processes. However, scientific validation of its anti-osteoporotic efficacy remains limited. Therefore, the present study aims to pharmacologically evaluate the anti-osteoporotic activity of *Curcuma caesia* extract using an ovariectomized rat model, mimicking postmenopausal osteoporosis. Parameters including bone mineral density (BMD), serum biochemical markers, and histopathological features will be assessed to elucidate its therapeutic potential.

MATERIALS AND METHODS

Materials

Fresh rhizomes of *Curcuma caesia* Roxb. were collected from a local herbal market. Ethanol (analytical grade) used for extraction was procured from Merck Life Science Pvt. Ltd., Mumbai, India. Dexamethasone and Raloxifene hydrochloride were obtained from Sigma-Aldrich, Bangalore, India. Carboxymethyl cellulose (CMC) was sourced from HiMedia Laboratories Pvt. Ltd., Mumbai, India, and used as a suspending agent. All chemicals and reagents used in the study were of analytical grade.

Methods

Extraction using maceration method

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials. 50 gram of *curcuma caesia* rhizome shade dried plant material were coarsely powdered and subjected to extraction with ethanol by maceration method (Mukherjee; 2007). Each extraction process was carried out for 24 hours. The filtrate was separated from the residue using Whatmann filter paper. The filtrate from each solvent was collected and evaporated using a water bath at 50°C until a thick extract was obtained. Finally, the percentage yields were calculated of the dried extracts.

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. % yield is calculated using the formula below:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Qualitative phytochemical screening

Qualitative phytochemical screening is carried out to investigate the various classes of natural compounds present in the extract. This is accomplished using standard methods (Tiwari *et al.,* 2011).

The classes of compounds identified in the extract included phenolics, flavonoids, tannins, saponins, alkaloids and protein.

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

b) Wagner test: two drops of Wagner reagent was added to 2 ml of extract and mixed well. Appearance of a reddish color indicates the presence of alkaloids.

2. Detection of Glycoside

a) Conc. H_2SO_4 Test: Extract dissolved in distilled water and treated with few drops of conc. Sulphuric acid. Formation of red color indicates the presence of glycoside.

3. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

4. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicate the presence of diterpenes.

5. Detection of phenols

a) Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

b) Folin Ciocalteu Test: 1 ml extract was added to 1 ml folin ciocalteu reagent; blue green color indicates presence of the phenols.

6. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

7. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

8. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates of saponins.

9. Detection of tannins

a) Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

10. Detection of Sterols

a) Salkowski Test: 3-4 drops of Conc. Sulphuric acid were added to the extract in chloroform. Formation of red color appears at the lower layer indicates the presence of sterols.

Experimental animals, housing and feeding conditions

Thirty female albino wistar rats were procured from the animal house of oriental Institute of Pharmacy. Rats were kept in a laboratory with an unlimited supply of drinking water and a temperature of 22°C (±3°C) and a relative humidity of atleast 30%. The exposure to light was 12 hours light, 12 hours dark; the room was lit for 12 hours per day (Rathi *et al.*, 2020). All the animals were grouped into six experimental group like:-

Group A - (2% CMC (Carboxy methyl cellulose) solution 5ml/kg),

Group B - (2% CMC) solution 5 ml/kg (ovariectomized rats),

Group C - (Raloxifene - 5.4 mg/kg i.p.)

Group D - (Ethanolic extract of *curcuma caesia* at a dose of 100 mg/kg),

Group E - (Ethanolic extract of at a *curcuma caesia* dose of 200 mg/kg).

Osteoporosis induction

The osteoporosis induction was done in thirty female wistar rats (six animals in each group) through the intramuscular administration of dexamethasone disodium phosphate (Decadron ® 4 mg/ml) at the dose level of 7 mg/kg of bodyweight, once a week for five weeks in all groups.

Blood sample collection, processing and analysis

After 30 days of the treatment period, all the rats were sacrificed, and blood sample was collected from the carotid artery. The serum calcium level, serum phosphorus levels were done by the standard methods.

Weight of femoral bone (gm)

The length was measured from the proximal tip of the femur head to the distal tip of the medial candyle using a digital caliper.

Estimation of bone calcium level

The bone mineral content was estimated by preparing left femur bone ash in a muffle furnace (700°C for 6 h) and dissolving it in a 0.1 mol/L HCl solution. Bone mineral (calcium) was measured by a UV-visible spectrophotometer.

RESULTS AND DISCUSSION

The present study evaluated the anti-osteoporotic potential of the ethanolic extract of *Curcuma caesia* rhizome in dexamethasone-induced osteoporotic rats, a model that mimics glucocorticoid-induced bone loss. The findings suggest that *Curcuma caesia* exerts a protective effect on bone health, likely through its phytoconstituents with known antioxidant and osteoprotective activities.

The percentage yield of the ethanolic extract was 8.25% w/w, indicating moderate extractive value from the rhizomes. Phytochemical screening (Table 2) revealed the presence of important bioactive compounds including alkaloids, flavonoids, diterpenes, phenols, proteins, and carbohydrates. These classes of compounds are known to contribute to bone health via different mechanisms. In particular, flavonoids and phenolic compounds possess strong antioxidant activity, which may help in reducing oxidative stress—a major factor contributing to osteoclast activation and bone resorption.

The biochemical analysis (Table 3) demonstrated a significant decline in serum calcium and phosphorus levels in the OVX control group (Group B) compared to the normal control (Group A), indicating effective induction of osteoporotic conditions. Treatment with *Curcuma caesia* at both 100

mg/kg and 200 mg/kg (Groups D and E) significantly restored these mineral levels, approaching values observed in the standard treatment group (Raloxifene, Group C). This suggests that *Curcuma caesia* may improve calcium and phosphorus metabolism or enhance their retention in bone tissue.

Assessment of bone parameters (Table 4) further supports the protective effect of *Curcuma caesia*. The OVX control group exhibited a notable reduction in femur weight, length, and bone calcium content, which are critical indicators of bone strength and quality. Treatment with the herbal extract significantly improved these parameters in a dose-dependent manner. Notably, the group treated with 200 mg/kg showed values nearly comparable to the raloxifene-treated group, indicating that *Curcuma caesia* might possess a bone-conserving effect similar to established anti-osteoporotic agents.

The observed pharmacological effects may be attributed to the synergistic action of multiple bioactive compounds. Flavonoids and phenolics could play a central role by modulating osteoblastic activity and suppressing osteoclastogenesis, while alkaloids and diterpenes might contribute to anti-inflammatory effects, reducing bone resorption.

Table 1: % Yield of *Curcuma caesia* rhizome extract

Sr. No	Extracts	% Yield (W/W)
1.	Ethanol	8.25%

Table 2: Result of phytochemical screening of *Curcuma caesia* rhizome extract

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Wagner's Test:	+ve
	Hager's Test:	+ve
2.	**Glycosides**	
	Conc. H_2SO_4 Test:	-ve
3.	**Flavonoids**	
	Lead acetate Test:	+ve
	Alkaline test:	-ve
4.	**Diterpenes**	
	Copper acetate Test:	+ve
5.	**Phenol**	
	Ferric Chloride Test:	+ve
	Folin Ciocalteu Test:	+ve
6.	**Proteins**	
	Xanthoproteic Test:	+ve
7.	**Carbohydrate**	
	Fehling's Test:	+ve

	Benedict's Test	+ve
8.	**Saponins** Froth Test:	-ve
9.	**Tannins** Gelatin test:	-ve
10.	**Sterols** Salkowski Test:	-ve

+Ve = Positive, -Ve= Negative

Table 3: Effect of Treatments on Serum Calcium and Serum Phosphorus in Dexamethasone-Induced Osteoporotic Rats

Group	Treatment	Serum Calcium (mg/dL)	Serum Phosphorus (mg/dL)
A	2% CMC (Normal Control)	10.5 ± 0.4	6.2 ± 0.3
B	2% CMC (OVX Control)	7.2 ± 0.5	4.1 ± 0.2
C	Raloxifene (5.4 mg/kg)	9.8 ± 0.3	5.9 ± 0.2
D	*Curcuma caesia* (100 mg/kg)	9.1 ± 0.4	5.6 ± 0.3
E	*Curcuma caesia* (200 mg/kg)	9.3 ± 0.3	5.7 ± 0.2

Table 4: Effect of Treatments on Bone Parameters in Dexamethasone-Induced Osteoporotic Rats

Group	Treatment	Femur Weight (g)	Femur Length (mm)	Bone Calcium (mg/g)
A	2% CMC (Normal Control)	0.96 ± 0.05	38.5 ± 1.2	180.2 ± 4.1
B	2% CMC (OVX Control)	0.61 ± 0.04	32.8 ± 1.0	121.4 ± 3.6
C	Raloxifene (5.4 mg/kg)	0.89 ± 0.03	37.2 ± 1.1	171.8 ± 4.5
D	Curcuma caesia (100 mg/kg)	0.81 ± 0.04	36.4 ± 1.3	165.0 ± 3.8
E	Curcuma caesia (200 mg/kg)	0.85 ± 0.05	36.8 ± 1.0	167.3 ± 3.5

CONCLUSION

In conclusion, the study demonstrates that ethanolic extract of *Curcuma caesia* rhizome significantly attenuates dexamethasone-induced osteoporotic changes in rats. These results support its traditional use and suggest its potential as a natural alternative for osteoporosis management. However, further studies involving bone histomorphometry, molecular mechanisms, and clinical evaluations are warranted to validate and expand on these findings.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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