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ScienceDirect

Journal of Acute Medicine 6 (2016) 7–10

www.e-jacme.com

Original Research

Evaluation of *Saraca indica* for the management of dexamethasone-induced osteoporosis

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Received 16 December 2014; revised 10 May 2015; accepted 2 September 2015
Available online 21 March 2016

Abstract

Objective: The aim of present study is to evaluate the effect of *Saraca indica* extract for the management of dexamethasone-induced osteoporosis in rats.
Methods: Female Wister rats were used for the investigation of antiosteoporotic activity of *S. indica* extract at the dose of 200 mg/kg/day, orally and various parameters such as alkaline phosphatase estimation, bone density, bone biomechanical strength, and ash value of bone were determined in dexamethasone-induced osteoporosis.
Results: Alkaline phosphatase level in the blood serum and ash value of the bone was found to be significant ($p < 0.05$) in the extract group as compared to the osteoporotic group while bone density and bone biomechanical strength of the femur bone of the extract group was found to be more than the osteoporotic group.
Conclusion: *Saraca indica* extract showed positive results in bone density, percentage ash value and biomechanical strength of rats.

1. Introduction

Ashoka is a Sanskrit word that means “without sorrow”. It is one of the most legendary and sacred trees of India. *Ashoka* tree is universally known by its binomial Latin name *Saraca indica* (Roxb.) De Wild or *Saraca asoca* belonging to family Caesalpinaceae. It is found throughout India and is used in many pharmacological activities, such as anticancer, antimenorrhagic, antioxytoxic, and antimicrobial activity, and has extended use in *ayurveda*, *unani*, and homeopathy.[1] In traditional literature, *ashoka* is reported to be used in leprosy,[2] wounds, snake bites, eye diseases, neurological, uterine disorder, fever, cough, and fracture of bones.[3] It contains, β-sitosterol, quercetin, kaempferol, epigenin, flavonoids, saponins, glycosides, sterols, tannins, calechor, octacosanol, epicatechin, procyanidin, catechin, glucosides, and leucocyanidine. *Ashoka* bark is traditionally administered in the form of a milk decoction.[4]

Corticosteroid-induced osteoporosis came into light when studies on Cushing syndrom e showed tendency of bone fracture due to excess of corticosteroid. There is a correlation between cumulative dose of corticosteroid and decrease in bone mineral density.[5] At higher concentration, corticosteroid reduces osteoblast activity and its differentiation. It blocks calcium absorption and increases activity of parathyroid hormone on osteoblast cells. It also affect messengers of bone and decreases secretion of gonadal steroids.[6]

Osteoblast cells are bone-forming cells and osteoclast cells are bone resorbing cells. Alkaline phosphatase (ALP) is a measure of osteoblast cells. Thus, the present investigation observed the change in ALP level, bone density, bone biomechanical strength and ash value of femur bone of female

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http://dx.doi.org/10.1016/j.jacme.2015.09.007

rats when treated with SI extract for dexamethasone-induced osteoporosis.

2. Materials and methods

2.1. Collection, authentication and drying of plant material

The bark of *Saraca indica* (SI) tree was collected from Kamla Garden, Bhopal district of Madhya Pradesh, India, identified and authenticated by botanist Dr Zia-ul Hasan at Safia College and a voucher specimen (No. 202/bot/safia/10) was submitted. Bark was washed, dried, and powdered. The powdered plant bark of SI was successively extracted with various solvents: petroleum ether, chloroform, ethyl acetate, and hydroalcoholic solution. All the above extracts were mixed together in order to obtain the whole extract of SI.

2.2. Phytochemical screening

In preliminary phytochemical screening, various chemical tests were performed for the identification of common phytoconstituents in SI extracts. The tests confirmed the presence of common constituents such as lipids, steroids, glycosides, flavonoids, tannins and phenolic compounds.[7,8]

2.3. Experimental animals

A total of 32 female rats weighing 100–150g were housed in the animal house in sanitized polypropylene cages containing paddy husk bedding. The animals were maintained under controlled conditions of temperature ($23 \pm 2^{\circ}C$), humidity ($50 \pm 5\%$), and a 12-h light–dark cycle. These rats were allowed free access to water and fed on a commercial diet. The study conducted were approved by the Institutional animal ethical committee (Registration No. 778/03/C/CPCSEA), VNS Institute of Pharmacy, Bhopal.

2.4. Acute toxicity

Various extracts of SI administered separately up to 2000 mg/kg body weight, none of the extracts produced any toxic symptoms of mortality, and hence the drugs were considered safe for further pharmacological screening.[9]

2.5. Model establishment

Antiosteoporotic activity was performed using dexamethasone-induced osteoporosis in female rats.[10] The animals were divided into four groups, eight animals in each group. Group 1 was kept as control group (positive control); Group 2 was treated with dexamethasone (negative control); Group 3 was treated with dexamethasone and standard drug sodium alendronate (0.2 mg/animal/day, orally); and Group 4 with dexamethasone along with mixed extracts (200 mg/kg/day, orally) of SI.

After 7 days of acclimatization, the female Wistar rats were given (except Group 1) dexamethasone sodium phosphate at 7 mg/kg (Decadron 4 mg/2 ml; Wockhardt, Mumbai, India) i.m. once a week up to 4 weeks. Weight of rats was observed during induction of osteoporosis and their treatment. A standard drug (sodium alendronate; 0.2 mg/animal/d orally) in Group 3, and mixed SI extracts (200 mg/kg) in Group 4 was administered for 15 days after 2 weeks of administration of Decadron.[3,11,12]

2.6. Analysis of parameters

2.6.1. Measurement of ALP

Blood samples of all rats were collected by orbital puncture of the left eye at the start and end of the protocol. Blood serum was separated from blood by cold centrifugation (Remi cooling centrifuge) at 1000 rpm for 20 min; 20 µL of serum was collected in Eppendorf tubes, 1000 µL of alkaline phosphatase reagent (Vital Diagnostics, PVT) was added in the same tube, and shaken; this process was carried out in dark and samples were analyzed in an auto analyzer ($Star^{plus}$).

2.6.2. Measurement of bone density

Air entrapped in the pores of bone was removed using a vacuum pump. Density of bone was calculated by calculating mass/volume of bone. To calculate volume of bone, bone was weighed; then it was transferred to conical flask filled with certain amount of water, then, weight of water was calculated, where weight of bone x, weight of water with bone w_2, weight of water $= (w_2 - x)$; therefore, volume of water $= (w_2 - x)/d$, (where d density of water). In another flask, the same amount of water was weighed (w_3) and volume of w_3 (v_3) was calculated using mass/density; the volume of bone was calculated using the following formula:

$$\text{Volume of bone } v_3 - (w_2 - x)/d$$

Finally, density of bone was calculated:

$$\text{Density of bone } = x/\text{volumeofbone}$$

2.6.3. Femur mechanical strength

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

2.6.4. Ash value

Ash value is the measure of inorganic contents of bone. Femur was kept at 600°C in a muffle furnace until the weight of bone became constant. The final weight of bone was measured.

2.7. Statistical analysis

The data were analyzed by using GraphPad prism software; one way analysis of variance was employed for the comparison of various experimental groups. A p-value < 0.05 was considered as statistically significant.