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Anti-osteoporotic activity of aqueous-methanol extract of *Berberis aristata* in ovariectomized rats

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ABSTRACT

Ethnopharmacological relevance: Traditionally *Berberis aristata* is employed for its supposed properties in treatment of joint pain and also used in alleviating symptoms of menopause.
Aim of the study: The aim of the present study is to evaluate the antiosteoporotic effect of *Berberis aristata* in ovariectomized (OVX) rats.
Materials and methods: Sprague–Dawley rats were divided into sham and OVX groups. The OVX rats were further divided into four groups, which received standard estrogen (0.0563 mg/kg) and 100, 300, and 500 mg/kg aqueous-methanol extract of *Berberis aristata*, daily for 42 days. The uterine weight, bone loss, ash content, biomechanical, biochemical and histopathological observation were carried out for antiosteoporotic activity.
Results: The experimental animals treated with *Berberis aristata* aqueous-methanol extract showed dose dependent activity. The significant increase in uterine weight, femur BMD, ash content and lumbar hardness were observed. In addition, increased levels of calcium and phosphorus in serum and significant decreased in urine were observed as compared to control OVX group. The histopathological results also confirm the protective effect of extract.
Conclusion: The present findings strongly suggest that *Berberis aristata* possess the potent antiosteoporosis activity in ovariectomized rats and substantiates the ethnic use in treatment of postmenopausal osteoporosis.

1. Introduction

Osteoporosis is most frequent major health hazard in recent years, affecting over 2000 million people world wide (Meryl, 1997) and characterized by low bone mass and microarchictectural deterioration of bone tissues, leading to enhanced bone fragility, which leads to a propensity to fracture with minimum trauma (Raisz, 2005). Menopause brings about increased bone turnover an imbalance between bone formation and bone resorption (Gruber et al., 1984). The most common type of osteoporosis is the bone loss associated with ovarian hormone deficiency at menopause (Riggs and Melton, 1986). Estrogen deficiency is considered as the main determinant for bone loss in postmenopausal women (Turner et al., 1994). Osteoporosis is caused by an imbalance in the normal bone remodeling process, in which there is excessive osteoclast resorption and adequate new bone formation by osteoblasts reduction. Hormone replacement therapy (HRT) has proven to be efficacious in preventing bone loss and reducing the incidence of skeletal fractures in postmenopausal women. However, long-term HRT increases the high risk of breast cancer, endometrial cancer, thromboembolic events and vaginal bleeding (Genunt et al., 1998). Traditional Indian medicines have been used from long days in prevention and treatment of postmenopausal osteoporosis. Since these medicines are prepared from medicinal plants they have fewer side effects and are suitable for long-term use.

Berberis aristata DC (Berberidaceae) known as 'Daruharidra' is a spine scent ethinomedicine, traditionally known for menopausal disorders and osteoporosis (Kulkarni and Shahida, 2004). *Berberis aristata* has been reported for its potential anti-platelet, antimicrobial and hepatoprotective activity (Chopra et al., 1996). The

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plant mainly contains berberine chloride, palmatine chloride, magnoflavine, canadine, berberastine, obaberine, columbavine and talifendine (Srivastava et al., 2001). In recent studies, berberine has been reported to possess various pharmacological effects including anticancer (Anis et al., 2001), and induction of apoptosis in several types of cells (Hwang et al., 2006). Berberine showed inhibition of bone resorption of ovariectomized rats and inhibited osteoclast formation in an *in vitro* cultured osteoblastic cell (Jing-Ping et al., 2008). Therefore, in our continuing screening of biologically active anti-osteoporotic agent using ovariectomized rat models, *Berberis aristata* DC aqueous-methanol extract was investigated.

2. Materials and methods

2.1. Chemicals and reagents

Calcium, phosphorous and alkaline phosphatase (ALP) kits were obtained from Erba diagnostics, Mallaustr, Germany. Estrogen and Ketamine obtained from Neon Laboratories Ltd., Mumbai, India. All other chemicals and reagents were of analytical grade.

2.2. Plant material and preparation of extract

Berberis aristata is widely available in India. The stem bark was collected in month of May–June 2008. It was identified and authenticated by Prof. S.A. Kappali, Botanist, Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka. The voucher specimen (Bsc/Pharmacy/12/2008) was deposited in Department of Pharmacology, Hangal Shri Kumareshwar College of Pharmacy, Bagalkot, Karnataka, India. The fresh and air dried stem barks were subjected to course powdering and passed through #44 mesh to get a uniform size. The powder (10 kg) was extracted with petroleum ether for defating and then by 1:1 ratio of aqueous-methanol (40–60 °C) for 24 h by using soxhlet apparatus, which yielded 0.67% brownish solid mass. The suspension of aqueous-methanol extract was prepared by using 5% Tween 80 and anti-osteoporotic activity studies were carried out in ovariectomized rats (Xie et al., 2005).

2.3. Phytochemical screening

Phytochemical screening of the aqueous-methanol extract of stem bark was carried out by employing standard procedures and tests (Trease and Evans, 1979), to find out the presence of chemical constituents such as alkaloids, terpenoids, flavonoids, tannins, coumarins, etc.

2.4. Animals

Forty eight female Sprague–Dawley rats (200–300 g) were used for the study. Animals were procured from the National Institute of Nutrition, Hyderabad and were quarantine for 10 days under standard conditions (temperature 21 ± 2 °C, relative humidity 55% ± 5%) for 12 h dark and 12 h light cycle, respectively, for acclimatization. They were given standard pellet food (Hindustan Lever, Bangalore, Karnataka) and water *ad libitum* throughout the experimental period. The study was approved by Institutional Animal Ethics Committee (IAEC-Clearance: 1-8/2007), Hanagal Shri Kumareshwar College of Pharmacy, Bagalkot, Karnataka.

2.5. Acute toxicity study

The acute toxicity study was performed as per the method described by Litchfield and Wilcoxon (1949), and LD50 was calculated accordingly. The aqueous-methanol extract of *Berberis aristata* in the dose range of 100–2000 mg/kg was administered orally to different groups of mice ($n = 10$). The animals were examined at every 30 min up to a period of 3 h and then occasionally for additional 4 h period, finally 24 h mortality was recorded. The antiosteoporotic activity was performed on female Sprague–Dawley rats at three dose levels 100, 300 and 500 mg/kg of body weight.

2.6. Experimental protocol of anti-osteoporosis activity

The Sprague–Dawley female rats were divided into 6 groups of 8 rats in each and treated as follows.

Group-I Sham operated receive vehicle (5% Tween 80);
Group-II Ovariectomized control receives vehicle (5% Tween 80);
Group-III Aqueous-methanol extract (100 mg/kg) in ovariectomized rats;
Group-IV Aqueous-methanol extract (300 mg/kg) in ovariectomized rats;
Group-V Aqueous-methanol extract (500 mg/kg) in ovariectomized rats;
Group-VI Estrogen (0.0563 mg/kg) in ovariectomized rats.

After seven days of acclimation, the rats were ovariectomized or sham operated. The rats were anesthetized with Ketamine HCl (50 mg/kg, i.p), and ovaries were removed bilaterally. Sham-operated animals were performed in same manner, but only exposing the ovaries. They were administrated with prophylactic gentamycine (10 mg/kg, i.p) for 3 days (Lasota and Danowask, 2004). The treatment of aqueous-methanol extracts *Berberis aristata* by oral gavages administration continued for 42 days. Body weight of all animals was measured weekly (Reddy et al., 2004). At the end of 42 days, all the rats were deprived of food for whole night. On the next day, urine (0–24 h) was collected, then the animals were anesthetized by Ketamine HCl (50 mg/kg, ip) and blood samples were taken from common carotid artery. The blood samples were centrifuged (MPW 350 R, Koria) at 2500 rpm for 15 min to separate the serum and preserved (−20 °C) for analysis of calcium, phosphorus and alkaline phosphatase (ALP) (Shirwaikar et al., 2003; Xie et al., 2005). Immediately, after collecting the urine and blood samples, uterus was carefully removed and weighed. The lumbar vertebra and femurs were isolated and stored at −70 °C until biochemical, biomechanical and histopathological studies were performed.

2.7. Femur physical parameter

Fresh isolated left femurs were weighed using an electronic scale. Length of the femurs was measured using a digital slide calipers. The length was measured from the proximal tip of the femur head to the distal tip of the medial candyle (Reddy et al., 2004). Bone volume and density were measured by fluid displacement method (Asankursekhar et al., 2005).

2.8. Lumbar compression test

The fourth lumbar vertebra was located and then it was isolated. The fresh vertebra was placed in digital hardness tester and compress until it got fractured and the reading was recorded in Newtons (N) (Shirwaikar et al., 2003).

2.9. Femoral ash weight, ash percentage and ash calcium

After measuring the bone length of left femur it was placed in tared fused silica crucibles and kept in Muffles furnace (Growell Instrument, Bangalore, India), dried to a constant temperature