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Ethnomedicine based evaluation of osteoprotective properties of *Tinospora cordifolia* on *in vitro* and *in vivo* model systems



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ABSTRACT

Indian ethnomedicine acclaims the use of Tinospora cordifolia (TC) in the treatment of bone fractures and vat rakta (gout). The objective of the study is to investigate the effects of alcoholic extract of Tinospora cordifolia on bone remodeling (involving osteoblastic and osteoclastic actions) in vitro and protect against ovariectomy-induced bone loss in vivo. Human osteoblast-like cells MG-63 and primary osteoblast cells isolated from rat femur were used as osteoblast models and RAW macrophage cell line 264.7 induced to take up osteoclastic lineage using RANK ligand were used as osteoclast models in the current study. Sirius red staining, quantification of osteocalcin, cytopathological analysis by Hematoxylin/eosin staining and semiquantitative reverse transcription PCR (RT-PCR) was carried out to ascertain the effects of T. cordifolia extract on osteoblast cells. MTT assay was perfored to understand the influence of T. cordifolia extract on osteoclast cells. Adult female Sprague-Dawley rats were used as in vivo models to study the effect of T. cordifolia on ovariectomy induced bone loss. Radiological (DEXA analysis), Biochemical (markers of bone formation and resorption), histopathological (Hematoxylin/eosin staining) and histomorphometric analysis of the bone was carried out. Treatment with T. cordifolia extract resulted in enhanced collagen deposition, increased levels of osteocalcin, increased expression of osteogenic genes all indicative of favourable osteoblastogenesis. Treatment with T. cordifolia extract did not exert any significant influence on the proliferation of osteoclasts. Pretreatment with T. cordifolia extract at a dose of 50 mg/kg body wt/day orally for 21 days followed by treatment for 12 weeks post ovariectomy was able to prevent ovariectomy-induced bone loss in vivo. Results of the study support the use of T. cordifolia in Indian ethnomedicine for the treatment of bone diseases and fractures.

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1. Introduction

Tinospora cordifolia (Willd.) Miers ex Hook. F. & Thoms, commonly known as Guduchi belonging to the family Menispermaceae is one of the most highly valued herbs in the Indian systems of medicine. Ayurveda refers T. cordifolia as "nectar of immortality (Amrita)" and "heavenly elixir". It is reported to be a health rejuvenator and immunomodulator [1]. In Indian ethnomedicine T. cordifolia was used in the treatment of fever, diabetes, dyspepsia, jaundice, urinary problems, skin diseases, chronic diarrhoea and dysentery. It has also been indicated useful in the treatment of heart diseases, leprosy, helminthiasis and

rheumatoid arthritis [2]. The muslim tribals of Rajouri, Jammu (Tawi) comprising Gujjar and Backwals used the plant in the treatment of bone fractures [3]. Various medicinal properties of *T. cordifolia* have been explored and scientifically documented. Some previous studies and several other ethnomedical/ethnopharmacological reports also highlighted the extensive use of the plant for the treatment of bone related diseases, especially in the healing of fractures [4,5].

One of the remarkable features of the mammalian skeleton is its ability to constantly renew itself throughout life which is referred to as "Bone remodeling". Bone remodeling equilibrium is maintained by a delicate balance in the action of bone forming osteoblasts and bone resorbing osteoclasts. Disruption of this equilibrium results in many pathological conditions including osteoporosis, osteoarthritis, periodontal disease, Pagets disease and osteopetrosis [6,7].

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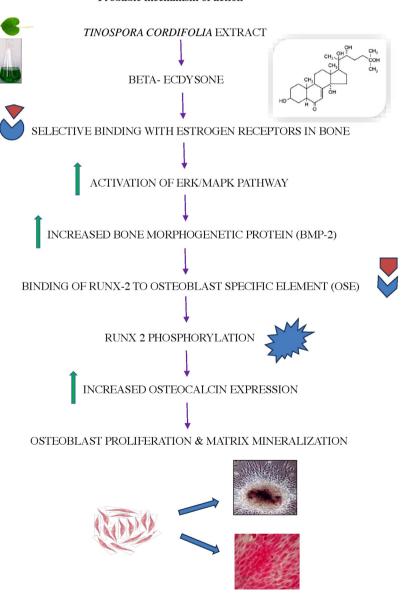
Inflammation is reported as one of the major causes for pathologies associated with the musculoskeletal, system [8]. Osteoporosis is a progressive systemic skeletal disease characterized by low bone mass, micro architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fractures. The condition is characterized by an increase in bone resorption relative to bone formation and a concomitant increase in the rate of bone turnover. Post-menopausal osteoporosis (PMO) is a progressive metabolic inflammatory bone disease in women associated with ovarian hormone deficiency following menopause. PMO is by and far the most common cause of age related bone loss in women.

As *T. cordifolia* is profoundly used in the preparation of several anti-inflammatory medicines and since it is recommended for the healing of fractures and inflammatory bone diseases in Indian ethnomedicine [9], it is more appropriate to validate the effects of *T. cordifolia* on bone remodeling (involving both osteoblastic and osteoclastic activity) *in vitro* and against experimentally-induced bone loss *in vivo*. This will provide a scientific rationale and justify the use of *T. cordifolia* in the Indian ethnomedicine for the treatment of bone diseases.

Previous studies from the laboratory [10] indicated the positive influence of *T. cordifolia* on osteoblastic proliferation/differentiation and thereby its effect on osteogenesis *in vitro*. This prompted us to investigate further about the effects of *T. cordifolia* on the deposition of collagen, levels of osteocalcin and on the expression of few important osteogenic genes. The effect of *T. cordifolia* on osteoclastic activity was investigated by performing cell growth and viability assays. The efficacy of the extract to stimulate osteoblast proliferation was compared with 17-beta estradiol to have a better understanding.

A study by Nandaa. [11] reported the clinical evaluation of a non-hormonal drug minofil containing *T. cordifolia* and compared its effectiveness with estradiol. The efficacy of *T. cordifolia* in treating post-menopausal symptoms might be closely related to its bone strengthening/protective properties. Although previous studies indicate the usefulness of the plant in treating bone disorders, a complete study on suitable *in vitro* and *in vivo* models is lacking. A thorough understanding of its effects on bone formation, its probable mechanism of action and identification of lead molecules responsible for its bone protective actions is lacking.

Probable mechanism of action



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