1 ABSTRACT

1.1 Background

Osteoporosis which means "porous bones," is a skeletal disease in which bones become brittle and prone to fracture. In other words, the bone loses density. Bone density is the amount of bone tissue (such as calcium and minerals) in a certain volume of bone. Osteoporosis is diagnosed when bone density has decreased to the point where the risk of fractures is high even without severe stress or injury to the bones. Many herbal drugs in India, has been traditionally used in Ayurveda to accelerate the healing of bone fractures and to strengthen the bones. However, no scientific study has been done to validate their usefulness in the alleviation of osteoporosis.

1.2 Aim

Investigation of some Ayurvedic herbal drugs for efficacy in post-menopausal osteoporosis using ovariectomized rat model.

1.3 Materials and method

In-vitro studies were performed on metaphyseal and diaphyseal femur bone to study effect of different extracts of Acacia arabica, Terminalia arjuna, Commiphora mukul and Boswellia serrata. The extracts (AAA, AAM, TAA and TAM) which showed significant effect in in vitro study were selected for in vivo studies using bilateral ovariectomized rat model. The animals were devided in to groups: Group I [SHAM Control (SHAM)], II [Disease control (OVX)], III [Standard estrogen (2 mg/kg/day/p.o.) (STD) ], IV [Aqueous extract of Acacia arabica (250 mg/kg/Body weight) (AAA250)], V [Aqueous extract of Acacia arabica (500 mg/kg/Body weight) (AAA500)], VI [Methanolic extract of Acacia arabica (250 mg/kg) (AAM250)], VII [Methanolic extract of Acacia arabica (500 mg/kg) (AAM500)], VIII [Aqueous extract of Terminalia arjuna (250 mg/kg/Body weight) (TAA250)], IX [Aqueous extract of Terminalia arjuna (500 mg/kg/Body weight) (TAA500)], X [Methanolic extract of Terminalia arjuna (250 mg/kg/ Body weight) (TAM250)], XI [Methanolic extract of
Abstract

*Terminalia arjuna* (500 mg/kg/ Body weight) (TAM500)]. TAM showed significant activity in in vivo study so, it was further fractionated to isolate active fraction. Bioactivity guided fractions of TAM (TAM-P, TAM-T, TAM-Et, TAM-Nbut, TAM-R) obtained by successive extraction with different solvents were evaluated using bone culture method. TAM-Nbut fraction was found to be most effective. Saponin (TAM-Nbut-S) fraction and aglycone fraction (TAM-Nbut-A) were isolated from TAM-Nbut and both the fractions showed significant activity in *in vitro* efficacy study. The aglycone fraction (TAM-Nbut-A) was found to contain arjunetin. Hence, arjunetin was evaluated for its efficacy on bone turnover. The content of arjunetin in aglycone fraction was estimated by HPLC.

1.4 Results

Macroscopical, microscopical and Phytochemical analysis of *Terminalia arjuna, Acacia arabica, Commiphora mukul* and *Boswellia serrata* confirmed their authenticity. The calcium content in the diaphyseal or metaphyseal tissues was significantly increased when the bone tissues were cultured in the presence of different extracts of *Terminalia arjuna* and *Acacia arabica*. Similar results were found with *Commiphora mukul*. However, extracts of *Boswellia serrata* did not show significant activity in *in vitro* bone-culture experiments. The methanolic extracts of *Terminalia arjuna* showed maximum anti-osteoporotic activity in *in vivo* study using ovariectomized rat model, among different extracts of Terminalia arjuna and Acacia arabica. TAM-Nbut fraction was found to be the most effective among all other fractions of TAM. Phytochemical screening showed the presence of saponins and tannins in n-butanol fraction of *Terminalia arjuna*. The saponin and sapogenin fractions from TAM-Nbut, significantly increased the calcium content in the metaphyseal tissues when the bone tissues were cultured in their presence. Arjunetin, which was one of the components of sapogenin fraction, showed significant effect on bone turnover. The content of arjunetin in TAM-Nbut- A was found to be 0.5790 %w/w.
1.5 Conclusion

The methanolic extract of *Terminalia arjuna* possesses significant anti-osteoporotic activity and arjunetin is one of the compound responsible for the activity. The possible mechanism of antiosteoporotic activity of *Terminalia arjuna* appears to be due to anti-inflammatory activity, anti-oxidant activity and phytoestrogen compound present in it.

1.6 Keywords

Post menopausal osteoporosis, Ovariectomy, Arjunetin , Saponin, *Terminalia arjuna*, *Acacia arabica*