Summary and Conclusion
Osteoporosis is a systemic skeletal disease that affects millions of people worldwide. Low bone mass, microarchitectural deterioration, increase in bone fragility and susceptibility to fracture are the hallmarks of osteoporosis. Several medications have been reported to be effective for treating osteoporosis. Many pharmacological agents are used to reduce fracture risk in osteoporosis. They include hormones, bisphosphonates and SERMs. Although these therapeutic agents effectively prevent post-menopausal bone loss and reduce fracture risks, safety is the major concern due to their undesirable side effects. As an alternative to these synthetic drugs, there is an increasing demand for ‘traditional system of medicine’ which could be healthier and safer for the treatment of osteoporosis and bone fractures.

Many plant products are used in the treatment of metabolic bone disorders including osteoporosis. *Cissus quadrangularis* (*C. quadrangularis*) is one of the important plant that has been used in the traditional system of medicine. Phytoestrogens present in *C. quadrangularis* possess anti-osteoporotic and fracture healing activities. However, the molecular mechanism behind these activities of *C. quadrangularis* is not well understood. The present study was performed to delineate the same using adult female Sprague-Dawley rats.

The fleshy stems of *C. quadrangularis* (2.5 kg) were air-dried and crushed into powder. The stem powder was exhaustively extracted with 95% ethanol and the extract yield of 225 g was obtained.

In the current study, unilateral closed fractures were produced in the left femur and the rats were divided into 2 groups (Fracture control and Fracture + *C. quadrangularis*). *C. quadrangularis* extract was given daily for 21 days from day 1
of induction of fracture. Animals from each group were killed on the post fracture days 7, 14 and 21. The callus tissue formed in the fractured femur was harvested on the post fracture days 7, 14 and 21. The mRNA and protein expression of a group of endogenous BMPs, VEGF, IGFs and their binding proteins in the fracture callus were investigated, using quantitative polymerase chain reaction (qPCR) and western blot analyses. Further the effects of *C. quadrangularis* on the antioxidant system and bone markers in the fracture callus of rats were also studied.

The mRNA expression of BMP-2, -4, -5, -6 and -7 were increased significantly at post fracture days 7, 14 and 21 in the femoral fracture callus of *C. quadrangularis* treated rats. The increase in the mRNA levels of BMP-2 and BMP-4 were accompanied by increase in their protein levels on post fracture days 7, 14 and 21. The mRNA expression of BMP-7 was unaltered on post fracture days 7 and 21. The protein expression of BMP-4 was unaltered on post fracture day 21 in *C. quadrangularis* treated rats. These results indicate the involvement of BMPs in regulating the effects of *C. quadrangularis* in bone fracture healing.

The mRNA expression of IGF-I and IGF-II was increased significantly on post fracture days 7, 14 and 21. The mRNA expression of IGF-II was unaltered on post fracture day 21. The increase in the mRNA levels of IGFBP-3, IGFBP-5 and IGF-IR were accompanied by increase in their protein levels on post fracture days 7, 14 and 21. An increase in the mRNA and protein expression of VEGF was observed on post fracture days 7 and 14. However, the mRNA and protein expression of VEGF was unaltered on post fracture day 21 in *C. quadrangularis* treated rats. These results indicate that increase in IGF components and VEGF by *C. quadrangularis* during specific time period could have facilitated the fracture healing.
*C. quadrangularis* treatment significantly combated the oxidative stress in the fracture induced rats. After fracture induction, there was increased TRAP activity and elevated levels of H$_2$O$_2$ and lipid peroxidation. Simultaneous administration of *C. quadrangularis* decreased the activity of TRAP, lipid peroxidation and H$_2$O$_2$ levels in the femoral fracture callus of rats. The activity of SOD, GPx, GST and ALP were significantly reduced in femoral fracture callus on post fracture days 7, 14 and 21. Interestingly, the activities of SOD, GPx, GST and ALP were significantly increased by *C. quadrangularis* treatment on post fracture days 7, 14 and 21.

In conclusion, the present study demonstrates for the first time that ethanolic extract of *C. quadrangularis* facilitates the femoral fracture healing process in rats possibly through the upregulation of BMPs, IGFs and their binding proteins and VEGF in the fracture callus. This study also reveals that the ethanolic extract of *C. quadrangularis* prevented the fracture induced oxidative stress in the femoral fracture callus. Taken together, the present study uncovers the mechanism that *C. quadrangularis* hasten the bone fracture healing process by up regulating the expression of certain growth factors in the fracture callus.

**Future direction**

The findings from the present study provide some valuable information on the effect of *C. quadrangularis* on bone fracture healing process in rats. *C. quadrangularis* contains vitamin C, β-carotene, calcium, flavonoids such as quercetin, genistein, luteolin and β-sitosterol. Incidentally, many of these constituents were already reported to have regulatory effects on some of the parameters in osteoblasts. Hence, the findings observed in the current study due to *C. quadrangularis* extract treatment were speculated to these principles. Future
studies with inclusive as well as exclusive regime for various principles of *C. quadrangularis* have to be conducted. This approach will help to specify the significance of each and every principle of *C. quadrangularis* in the fracture healing process.

In order to prove that *C. quadrangularis* mediates its healing activities through certain growth factors, studies on conditional knockout of their genes and the subsequent treatment with *C. quadrangularis* are warranted.