

**CHAPTER 2****EFFECT OF *MORINGA OLEIFERA* PLANT EXTRACT ON OVARIECTOMIZED  
HYPERGLYCEMIC RATS**

Diabetes mellitus is a metabolic disorder associated with several complications, including impaired healing. Bone, as important skeletal structure in the body, is affected by the diabetic condition, particularly during fracture healing processes. Bone is a tissue that undergoes frequent remodeling and has a large capacity for regeneration. In the adult remodeling occurs so that the skeleton is replaced approximately every 10–11 yr (Parfitt, 1982; He *et al.*, 2004). This physiologic remodeling is initiated by osteoclasts that resorb bone and is followed by the formation of an equivalent amount of new bone by osteoblasts (Mundy, 1989). Bone loss is noted when the amount of bone resorption exceeds the amount of new bone formation. This occurs in the aging skeleton, especially during menopause related osteoporosis (Hayward and Fiedler, 1987).

Diabetes has also been associated with a net loss of bone. A number of studies have reported that type 1 diabetes alters bone remodeling by reducing the formation of new bone, leading to osteopenia. This has been shown by a decrease in bone mineral density in humans and alterations in the formation of new bone in animal studies (Levin *et al.*, 1976; Horcajada-Molteni *et al.*, 2001; Tuominen *et al.*, 1999; Krakauer *et al.*, 1997). It has previously been noted that there is reduced fracture healing or osseous repair after marrow ablation in diabetics, compared with normal (Herskind *et al.*, 1992; Gooch *et al.*, 2000; Kawaguchi *et al.*, 1994 and Lu *et al.*, 2003). Several mechanisms have been proposed including diminished production of growth factors and expression of transcription factors that regulate osteoblast differentiation. We have found that OVX hyperglycemic rats did show some alterations in the biochemical as well as the histomorphological features (Chapter I). Diabetes may have a general effect on increasing apoptosis of matrix-producing cells, which limits the repair of injured tissue (He *et al.*, 2004).

Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries

because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Modak *et al.*, 2007), they have given the list of medicinal plants with proven anti-diabetic and related beneficial effects and of herbal drugs used in treatment of diabetes is compiled. This includes *Allium sativum*, *Eugenia jambolana*, *Momordica charantia*, *Ocimum sanctum*, *Phyllanthus amarus*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Trigonella foenum graecum* and *Withania somnifera*. Over and above number of scientists have explored the anti-diabetic properties of *Elephantopus scaber* L. (Daisy *et al.*, 2009), *Ligustrum lucidum* (Dawei *et al.*, 2007); *Momordica charantia* fruit extract (Fernandes *et al.*, 2007); antidiabetic and antioxidative effects of *Annona squamosa* leaves (Panda and Kar, 2007). Tan and his team (2008) has proven the anti-diabetic activities of triterpenoids isolated from bitter melon.

Similarly, a large number of studies have proved the effectiveness of botanicals in improving bone health. The literature study of osteoporosis reveals that garlic oil, 1, 25 dihydroxy vit-D<sub>3</sub> and calcium in osteopenic ovariectomized rats has been performed by Rasheed and coworkers (2009). Efficacy of OST-6, a polyherbal formulation in the management of osteoporosis in postmenopausal women is also reported (Ahmed *et al.*, 2002). *Withania somnifera* is a rejuvenator that helps in relieving pain associated with osteodystrophic conditions (Mitra *et al.*, 2000) and is also useful in people with general debility, nervous problems and muscular pain. *Commiphora wightii* increases the mineralization of bones (Putnam *et al.*, 2007; Kulhari *et al.*, 2012). The paste of leaves and fruit is applied to relieve the pain associated with bone fracture in animals (Khan, 2009). Bamboo leaves possess astringent activity, which causes early regression of inflammation that promotes fracture healing. Bamboo also contains benzoic acid, traces of cyanogenic glycoside which helps in fracture healing. Besides these it also contains calcium, phosphorus and zinc which used orally or topically for healing fracture (Asgar, 1999; Jaiswal *et al.*, 2004). *Cissus quadrangularis* Linn, contains calcium oxalate, Carotene, vitamin, Terpenoids, sitosterols, which are responsible for acceleration of bone healing and possess anti-inflammatory and analgesic activity (Prasad and Udupa, 1972; Asgar, 1999; Sarvanan *et al.*, 2002; Deka and Lohan, 1993). Root paste of *Symphytum officinale* Linn helps in the union of fracture bone (Gaur *et al.*, 1992). *Sida cordifolia* contains phytosterol and potent phytoestrogens and has been given in a herbomineral formulation osteoporosis (Shah and Kolhapure, 2004;

Ahmed *et al.*, 2002; Dongaonkar *et al.*, 2005). *Vanda roxburghii* has anti-inflammatory activity, which is useful for relief of bone pains of osteoporosis (Reddy and Kulkarni, 2002; Sharma and Srivastava 2003).

From the above literature survey one can see that there are plenty of herbals for diabetes and osteoporosis at individual level, but very few are having positive effect both on carbohydrate as well as mineral metabolism. One of such plant is *Moringa oliefera* (MO), which has been nicknamed as a “wonder tree” as the benefits of MO is plentiful: Practically every part of the tree is beneficial in some way. The use of various parts of the Moringa tree is widespread all over the globe and the multipurpose nature of the tree can be seen in the wide range of uses of its parts for food, fodder and medicinal use. MO leaves are an excellent source of vitamins (especially Vitamin A, B and C), minerals (calcium, iron) and protein. They are used to combat malnutrition, blindness, diabetes, high blood pressure, anemia, urinary tract problems, kidney stones, to induce lactation in nursing women, and as an antiseptic. The multipurpose nature of the plant makes it understandable that it is often referred to as a “wonder-tree”. Immense work has shown the anti-diabetic property of MO plant is because of MO flavonoids, especially Q3G, which affect glucose uptake by intestinal mucosa and therefore affect the time course of appearance of glucose in the blood and their availability to other parts of the body (Ndong *et al.*, 2007). Jaiswal and co workers (2009) have scientifically validated the widely claimed use of MO as an ethnomedicine to treat diabetes mellitus. Chen and his colleagues (2007) in their studies have shown that leaf flavones remarkably decreased the serum glucose; improve activity of serum SOD in alloxan-induced diabetic mice. Dièye *et al.*, 2008, Gupta *et al.*, 2010 have also worked on MO but not as a hypoglycemic agent. Studies conducted at our lab established one more pharmacological effect of this plant as an osteoprotective agent (Rangrez *et al.*, 2011).

From the findings of the previous study (Chapter I) it was concluded that hyperglycemia is having deleterious effects on OVX induced bone loss. It was learned from the literature survey that MO is rich in variety of anti-oxidants, polyphenols and flavonoids which have positive effect, both on bone and diabetes. Hence, we tested this plat for one more step further to explore its efficacy as an osteoprotective agent in hyperglycemic OVX rats. Hence, the aim of the

present study was to explore the preliminary effect of MO plant components for their effect on the bone health markers in hyperglycemic OVX rats.

**Materials and Methods:**

Fruits, leaves and flowers of MO were obtained from the field and their herbarium sheet was submitted in the botany department for species verification. Dried powder was prepared by drying MO in oven at 50° C. 100 gm dried powder of each component was extracted with 500 ml methanol in Soxhlet's apparatus for 48 hours . Methanolic extract was dried on water bath at 55° C. The percentage yield of the plant was found to be 9.8%, 6.3% and 7.7% for fruits, leaves and flowers respectively. The plant extract was freeze dried and stored at -70° C. Working solution was prepared by dissolving the extract in saline.

Healthy adult female Wistar rats (80–90 days old) were maintained in a well ventilated, temperature-controlled room on a 12 h light: 12 h dark schedule. The rats were fed with standard balanced rat pellets (Baroda., India) and drinking water was made available *ad libitum*.

The rats were divided into the following groups: (i) Control (C) (ii) Streptozotocin induced diabetic (iii) Ovariectomized (OVX) (iv) Streptozotocin induced diabetic + Ovariectomized (v) hyperglycemic ovx + plant extract. Each group consisted of 5 rats. Induction of diabetes and OVX was induced as described in Chapter I.

The control group was given only the vehicle. Group V was further subdivided into three groups, which were given the leaf, flower and fruit extract treatment at 200mg/kg bodyweight. After 30<sup>th</sup> day the animals were sacrificed and serum glucose, ALP. And TRAcP content was measured in the serum.

**Results:**

Our results showed that this plant is having ameliorating effect on the changes which are induced due to estrogen loss and STZ injection (Table 1). Figure 1 shows the reduction in the blood glucose levels of DP rats following MO treatment. Though the data was statistically non significant for leaf and flower extract, one can see the protective effect of MO components. Fruit

extract was found to be significant in reducing the elevated glucose levels in hyperglycemic OVX rats (Figure 1).

In response to the exposure of different component of MO, the ALP activity too showed a promising result, though leaf extract exposed OVX hyperglycemic rats showed a non significant decrease, whereas the Flower and fruit extract exhibited significant decrease in the serum ALP levels (Figure 2).

Similarly, TRAcP levels, important markers of increased osteoclastic activity during osteoporotic like condition, were seen to be significantly decreases only on exposure to the flower extract, and the leaf and fruit extract did show decrease however, the decrease was non-significant (Figure 3).

### **Discussion:**

The physiologic remodeling is initiated by osteoclasts that resorb bone and are followed by the formation of an equivalent amount of new bone by osteoblasts. Bone loss is noted when the amount of bone resorption exceeds the amount of new bone formation. This occurs in the aging skeleton; especially during menopause related osteoporosis (Hayward and Fiedler, 1987). Diabetes has also been associated with a net loss of bone. A number of studies have reported that type 1 diabetes alters bone remodeling by reducing the formation of new bone, leading to osteopenia. This has been shown by a decrease in bone mineral density in humans and alterations in the formation of new bone in animal studies (Levin *et al.*, 1976; Krakauer *et al.*, 1995; Tuominen *et al.*, 1999 and Horcajada-Molteni *et al.*, 2001). The impact of type 1 diabetes on bone is reflected by a significant delay in fracture healing (Macey *et al.*, 1989 and Gebauer *et al.*, 2002). Tuominen *et al.* (1999), ha opined that the reduced bone mass in type 1 diabetics was due to a higher rate of bone loss, and that type 1 diabetes has a more profound effect on physiologic bone remodeling than does type 2 diabetes.

In animal models, type 1 diabetes is associated with bone loss, decreased osteoblast activity, increased bone marrow adiposity, osteoblast death and marrow inflammation (Coe *et al.*, 2011 and McCabe, 2007). Reports using type 1 diabetic animal models have also demonstrated that animals display bone loss regardless of method of diabetes induction i.e. spontaneous versus pharmacologic (Botolin and McCabe, 2007). However, differences in mouse genetic

backgrounds did affect the magnitude of bone loss in response to type 1 diabetes, possibly as a result of differences in bone turnover rates and/or additional metabolic factors as approved by Kar *et al.*, 2003, where they have given the ethanolic extract and found that alloxan induced diabetic rats showed significant blood glucose lowering effects, and reverse to this was the result of Mossa, (1985) who showed that MO increased the blood glucose by 15% in alloxanized mice.

Both type 1 and type 2 diabetes lead to increased fracture risk (Shivaswamy *et al.*, 2011), making diabetic bone loss a complication that should be considered for monitoring and therapeutic prevention. First line approaches include exercise/loading of bones, vitamin D and calcium to enhance and/or maintain bone density. Given that most pharmacologic approaches for bone loss reduce catabolic activity by osteoclasts and knowing that diabetes (especially type 1) can suppress catabolic and anabolic properties, diabetic treatment options need to be considered carefully.

Recent research has now indicated that these herbs are a rich source of phenolic phytochemicals having high antioxidant activity. Further it has shown that these phenolic phytochemicals possess specific therapeutic properties and may be responsible for their beneficial effect on human health (Hertog *et al.*, 1995 and Paganga *et al.*, 1999). Phenolic phytochemicals are now implicated to have potential for management of many chronic oxidation-linked diseases, one of them is diabetes (Jadeja *et al.*, 2010). MO leaves significantly decrease blood glucose concentration in Wistar rats and Goto-Kakizaki (GK) rats, modeled type 2 diabetes (Ndong *et al.*, 2007). Another study indicated that the extract from Moringa leaf is effective in lowering blood sugar levels within 3 h after ingestion (Mittal *et al.*, 2007). Moringa leaves are potent source of polyphenols, including quercetin-3- glycoside, rutin, kaempferol glycosides, and other polyphenols (Ndong *et al.*, 2007). All the above mentioned work has been done in diabetes alone, ours is the first report to authenticate that the fruit, flower and the leaf of MO has the potential for reducing the glucose titre in OVX hyperglycemic rats, the probable mechanistic pathway is due to the presence of the flavanoids and the polyphenols, as reported and proposed by (Grassi *et al.*, 2005; Al-Awwadi *et al.*, 2004; Moharram *et al.*, 2003) are responsible for hypoglycemic activity.

The AIP and the TRAcP are the specific markers for the bone formation and bone resorption. MO plant have been proved to possess the osteoprotective potency, hence, in an attempt to show

the osteoprotective as well as the hypoglycemic efficacy of the plant, serum glucose, serum ALP and TRAcP levels were monitored. To our surprise, of all three components, leaf extract did not exhibit any significant change in the titer of these markers. Compared to leaf, both flower and fruit showed osteoprotective effect on the ALP and TRAcP. These two are the established marker for studying bone formation and bone resorption in vivo. Our study showed that these parameters are affected in hyperglycemic OVX rats and treatment with MO flower and fruit extract, one can ameliorate these changes. Furthermore, the TRAcP activity, marker of intense osteoclastic resorption was more potently inhibited by flower extract, which may be due to presence of variety of antioxidants and flavonoids which are present in the flower. It has already been established that flavonoids have anti osteoclastic activity and as flower is rich in these flavonoids, one can understand the potent effect of flower extract on this osteoclastic function marker.

Though established as multipotent pharmacological botanical, very few reports are available explaining the osteoprotective efficacy of MO in OVX rats (Burali *et al.*, 2010, Rangrez *et al.*, 2011), who have opined that MO may have protective action against ovarian hormone insufficiency – related bone resorption. In another study by Choi *et al.*, (2001) proved that phytoestrogens perform their anti-osteoporotic effect by stimulating osteoblastic activity through an estrogen receptor mediated action, or by increasing the production of insulin like growth factor-1 (IG-F) which is known to enhance osteoblastic activity. And positively affect bone mass in osteoporotic subjects, confirming that it is the polyphenols present in the MO which is a probable agent. Phytochemical screening has also proves the presence of the polyphenol compounds in MO plant. Thus, it can be claimed that ours is the first report which has confirmed that Different components of MO plant exhibits differential expression for its osteoprotective potency on OVX Hyperglycemic rats.

In conclusion, our study provided the first insight into the probable effect of MO and its different components on hyperglycemic OVX rats and showed that consumption of this plant may have beneficial effect in ameliorating the deleterious effect of these two diseases.

***However, during this study we have undertaken several parameters of glucose metabolism, bone function marker and histology, which did not give clear results. Markers of liver health***

*like SGOT, SGPT, ALP and markers of Kidney functions like urea and creatinin clearance rate were not related with the MO treatment. This is probably because of the complex interrelation of these two metabolic disorders and hence, all further studies were planned to explore MO and its components further for its osteoprotective effect in vivo and in vitro.*



Table 1: Showing serum Biochemical markers in different treatment groups.

<b>Serum profile</b>	<b>Control</b>	<b>Diabetes</b>	<b>Osteoporosis</b>	<b>Hyperglycemic OVX</b>	<b>Hyperglycemic OVX + leaf</b>	<b>Hyperglycemic OVX +flower</b>	<b>Hyperglycemic OVX + fruit</b>
Blood glucose	122.95 ± 5.07	422.02*** ± 25.07	142.392 ± 10.87	386.134*** ± 65.20	329.122 ± 45.36	288.36 ± 61.39	211.560* ± 61.538
ALP level	59.760 ± 7.28	54.401 ± 8.06	104.698*** ± 7.53	96.163*** ± 6.26	76.880 ± 5.06	70.255* ± 70.32	66.548** ± 5.217
TRAcP	5.999 ± 1.37	6.661 ± 2.88	13.698*** ± 2.87	10.705* ± 2.59	9.520 ± 1.65	6.448* ± 2.83	8.996 ± 1.395

Values were expressed as Mean ± S.E.M. \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ .

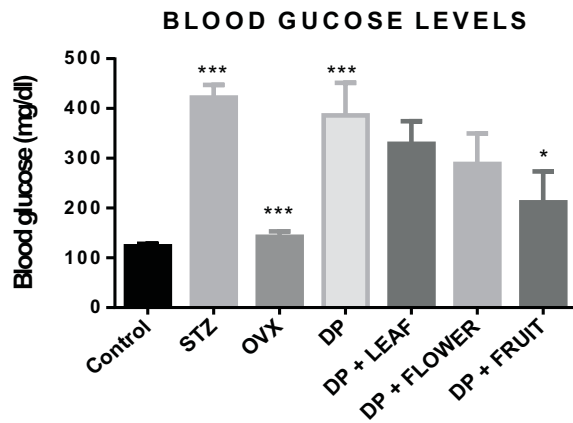


Figure 1

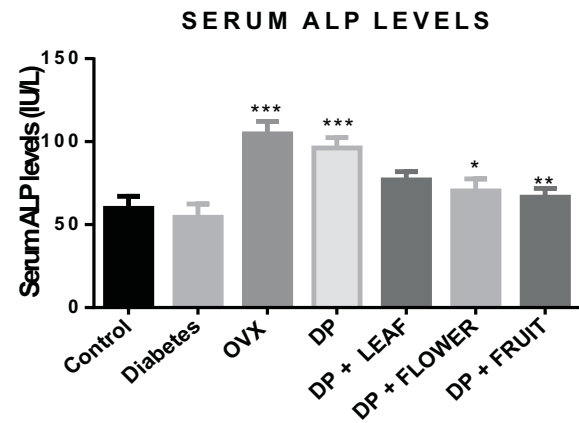


Figure 2

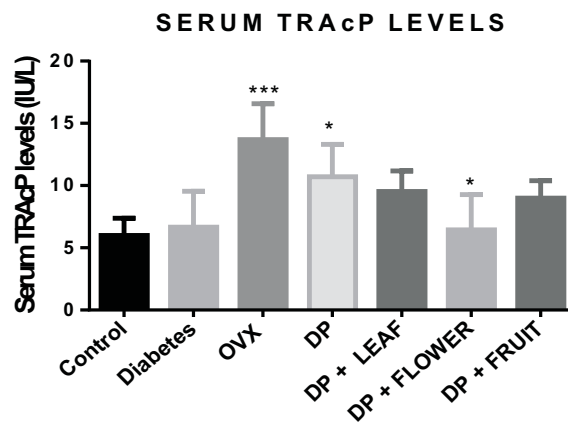


Figure 3

Figure 1: Decrease in blood glucose following plant treatment; Figure 2: Decrease in serum Alp activity following plant treatment Figure 3: Decrease in serum TRAcP activity following plant treatment