Studies on the Osteoprotective and Antidiabetic Activities of *Moringa Oleifera* Plant Extract.

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ABSTRACT: *Diabetes mellitus has been known to be associated with a high risk of osteoporosis. Moringa oliefera , a traditional Asian herbal medicine, has various uses, such as antioxidants, antiaging and anti-inflammatory treatment, among others. We have investigated the effect of different parts of Moringa oliefera (Leaf, fruit and flower extract) on bone health markers in diabetic osteoporosis (STZ-OVX) rats. MO treatment resulted into a reduction in glucose levels in STZ-OVX rats, of the three components fruit extract was found to be highly significant in reducing the elevated glucose levels as well as osteoclastic bone marker TRAcP in STZ-OVX rats. On the other hand it resulted into a increase in ostoblastic marker ALP in STZ-OVX rats. Of all the three parts of MO exposed it was fruit which exhibited the maximum amelioration. The results obtained in the present study provide evidence that MO fruit extracts contributes importantly to the prevention of bone loss in STZ-OVX rats.*

Key words: ALP, Diabetes, Glucose, Moringa olifera, Osteoporosis, TRAcP.

I.   Introduction

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. 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 (1). It has previously been noted that there is reduced fracture healing or osseous repair after marrow ablation in diabetics, compared with normal (2, 3). Diabetes may have a general effect on increasing apoptosis of matrix-producing cells, which limits the repair of injured tissue (4). Effect of hyperglycemia on ovariectomy induced bone loss has also been reported by (5). 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.(6) in their studies have compiled and listed herbals used in treatment of diabetes, 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.* (7), *Ligustrum lucidum* (8)*; antidiabetic and antioxidative effects of Annona squamosa* leaves (9,10) have 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 viz., *Withania somnifera* a rejuvenator that helps in relieving pain associated with osteodystrophic conditions (11) ; *Commiphora wightii* increases the mineralization of bones (12,13); *Litsea glutinosa*, an ayurvedic herb, has been reported for its effects on ovariectomy induced bone loss, calcium metabolism, and prevention of osteoclastic bone resorption (14,15).

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 diabetes and osteoporosis. *Moringa oliefera* (MO), which has been nicknamed as a "wonder tree" because the benefits of MO are plentiful. Anti-diabetic property of MO plant has been proved by (16,17,18).   While, (19) have established pharmacological effect of MO plant as an osteoprotective agent. MO is rich in variety of anti-oxidants, polyphenols and flavonoids which have positive effect, both on bone and diabetes. Hence, the aim of the present study was to explore the efficacy of MO plant components for their effect on the bone health markers in STZ- OVX rats.

II. Materials and Methods:

2.1 Preparation of Extract: Fruits, leaves and flowers of MO were obtained from the field and their herbarium sheet was submitted in the Botany Department, The Maharaja Sayajirao University, Baroda 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.

2.2 Experimental model: 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 (STZ) (iii) Ovariectomized (OVX) (iv) Streptozotocin induced diabetic + Ovariectomized (STZ-OVX) (v) STZ-OVX + plant extract. Each group consisted of 6 rats. 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 (LD_{50}=400mg/kg B.W).

2.3 Biochemical assays: After 30th day the animals were sacrificed**,** Blood glucose, ALP and TRAcP activity in serum was estimated using a commercially available kit (Reckon Diagnostics, India).

2.4 Statistical Analysis: One Way ANOVA followed by multiple comparison of Data obtained were performed by using Graph Pad prism 5 (p values < 0.05 was considered significant).

III. Results:

Effects of different components of MO are presented in Table 1 and Figure a, b, c. MO treatment resulted into a reduction in glucose levels in STZ-OVX rats, however, of the three components fruit extract was found to be highly significant in reducing the elevated glucose levels in STZ-OVX rats. In response to the exposure of different component of MO, the AlP activity too showed a promising result, MO extract exposed STZ-OVX rats showed a significant decrease, in which the fruit extract exhibited more significant decrease in the serum AlP levels. Similarly, TRAcP levels, important markers of osteoclastic activity during osteoporosis was seen to decreases more significantly only on exposure to the fruit extract than that of the leaf and flower extract .

IV. Discussion:

Diabetes mellitus is a pandemic metabolic disease with substantial morbidity and mortality. Patients with diabetes mellitus have various skeletal disorders, including osteopenia or osteoporosis, Charcot's arthropathy, and the diabetic foot syndrome (20). In principle, bone and mineral abnormalities in patients with diabetes mellitus may be caused by direct effects of insulin deficiency or resistance and hyperglycemia on the bone and bone marrow microenvironment, advanced glycosylation end products of bone matrix proteins, abnormal cytokine and adipokine production and their detrimental effects on bone cells, and impaired neuromuscular/skeletal interactions (21, 22). Recent research has now indicated that MO is a rich source of phenolic phytochemicals and posses numerous therapeutic properties (23, 24, 25). Phenolic phyto chemicals are now implicated to have potential for management of many chronic oxidation-linked diseases; one of them is diabetes (26). MO leaves significantly decrease blood glucose concentration in Wistar rats (17). Another study indicated that the extract from MO leaf is effective in lowering blood sugar levels within 3 h after ingestion (27). MO leaves are potent source of polyphenols, including quercetin-3- glycoside, rutin, kaempferol glycosides, and other polyphenols (17). 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 STZ-OVX rats. The AlP and the TRAcP are the specific markers for the bone formation and bone resorption respectively. MO plant have been proved to possess the osteoprotective potency (5), 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. Compared to control and OVX there was a significant increase in the diabetic (STZ induced) group due to insulin resistance or due to malfunctioning of Glut receptors. OVX hyperglycemic rats also maintained the same physiological glucose load. Treatment with MO extracts resulted into a reduction in the glucose load; however, due to the synergistic effects of osteoporosis as well as hyperglycemia, it did not reduce to the normal level of that of control. This can be probably due to a negative calcium balance as an outcome of both enhanced urinary calcium loss and depressed intestinal absorption, itself perhaps a consequence of reduced renal synthesis of 1, 25-dihydroxy vitamin D_3 (5). Another reason for decrease in glucose titer can be attributed to the role of the flavanoids and the polypenols, present in different

components of MO extracts (28, 29). AlP and TRAcP are the most useful markers of studying bone formation and bone resorption, respectively (30, 31, 32). In the present studies the TRAcP levels were seen to be decreasing compared to the STZ-OVX group suggesting the amelioratiing effect of the MO extract. Furthermore, the decreased TRAcP indicates a fall in osteoclasts activity and decline in bone resorption. Increased TRAcP level in OVX group is as expected, as OVX results into loss of estrogen which plays important role in bone metabolism through secretion of osteoprotegrin (33,15). In absence of estrogen, there is aggravated osteoclastogenesis, leading to more bone resorption. Parallel to the TRAcP levels, reverse was observed in ALP levels. Compared to control STZ, OVX and STZ-OVX all showed a significant increase in the ALP levels. Higher AlP level in the OVX rats have been correlated with the increased bone turnover rate (15,16), which suggest that bone remodelling in rats is accelerated after ovariectomy. Furthermore, increased ALP in OVX group has been correlated due to lack of inhibiting activity of estrogen on Osteoclasts causing the increase in bone resorption (32). Simultaneously with intensification of resorption process, the bone formation process was also increased by enhancement of osteoblast activity, which led to enhancement of ALP activity in the OVX group (30,16,31), whereas in MO extract treated groups the ALP activity was less than STZ- OVX, suggesting improved the activity of alkaline phosphatase, the messenger initiating calcification, it is possible that the herb accelerated mineralization of the organic matrix, thus speeding up the bone

V. Conclusion

Our study provided the first insight into the probable effect of MO and its different components on STZ- OVX rats and showed that consumption of this plant may have beneficial effect in ameliorating the deleterious effect of these two diseases. The results obtained in the present study provide evidence that MO fruit extracts contributes importantly to the prevention of bone loss in STZ-OVX rats. However, in order to develop MO plant extract in the international scientific community as an alternative regime for the treatment of bone diseases, more research will be needed to identify the active ingredients in MO plant extract as well as the mechanism that mediates the action of MO plant extract in vivo.

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

Serum profile	Control	Diabetes	Osteoporosis	STZ- OVX	STZ- OVX + leaf	STZ-OVX +flower	STZ-OVX + fruit
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** ± 7.32	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

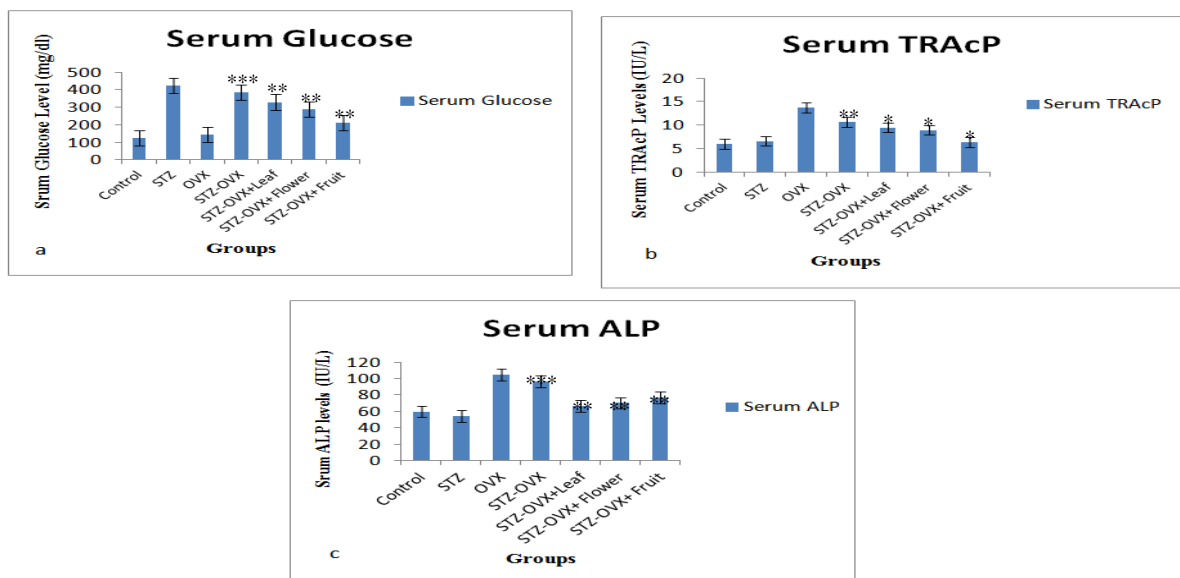Values were expressed as Mean ± S.E.M. * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

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Figure: a, b and c showing the Serum Glucose, ALP and TRAcP levels.

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