CHAPTER 2

OSTEOPROTECTIVE EFFECT OF LG IN OVARIECTOMIZED WISTAR RATS

Our previous study (Chapter 1) has shown that LG is having positive effect on bone. Hence, a further study was designed to explore the osteoprotective effect of *LG* in OVX wistar rats.

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INTRODUCTION

Postmenopausal osteoporosis is a major age related health problem for women who often have negative calcium balance due to decrease in intestinal calcium absorption, insufficient dietary calcium intake, as well as increase in urinary calcium loss associated with estrogen deficiency during menopause. Osteoporosis is a progressive debilitating process that reduces cancellous bone and leads to the weakening of the overall integrity and stability of bone. This result in enhanced bone fragility and a consequent increase in fracture risk (Nozaka *et al.,* 2008). The pathogenesis of postmenopausal osteoporosis is manifested by a drop in ovarian estrogen levels after menopause and increased bone resorption. This increase in bone resorption by osteoclasts resulting in decreased bone mass is also associated with an increase in the production of pro-inflammatory cytokines such as IL-1 and IL-6. These pro-inflammatory cytokines contribute to bone resorption by increasing osteoclastogenesis (Xie *et al.,* 2005; Park *et al.,* 2008).

Until recently, calcium supplementation with vitamin D and HRT with estrogen, selective estrogen receptor modulators, calcitonin, raloxifene, amino-bisphosphonates, teriparatide, parathyroid hormone, strontium ranelate, growth hormone, and insulin like growth factor - 1(IGF -1) were the mainstays in the treatment of menopause associated osteoporosis (Park *et al.,* 2008). Recent evidence suggest that estrogen replacement therapy (ERT) is associated with increased risk of breast, ovarian and endometrial cancer in postmenopausal women, it is now generally recognized that alternative approaches to the prevention and treatment of osteoporosis might be worth exploring (Xie *et al.,* 2000). That is why recently the attention has been focused on phytoestrogens and phytotherapy for the treatment of osteoporosis.

The ovariectomized rat model is a scientifically accepted model of osteoporosis. The various pathological processes found in this model are similar to those found in humans. In both species bone loss is most rapid after the onset of estrogen deficiency. This is characterized by a period of increased bone turn over during which resorption exceeds formation. In both species, bone loss from trabacular bone is greater than cortical bone (McCann *et al.,* 2005). According to Dontas *et al.,* (2006) also the ovariectomy is a consistent and reproducible model used in skeletal research. These similarities are strong evidence that the ovariectomized

(OVX) rat bone loss model is suitable for studying the prevention and treatment of postmenopausal bone loss (Xiao *et al.*, 2002).

Indian herbal medicine has been widely used for the treatment of many diseases from thousands of years. There are numerous plants which have been shown to have positive effect on bone remodeling and few of them are used in the prevention of osteoporosis. *L. glutinosa* commonly known as "Maida Lakri" is said to be one of the most potent plants for treatment of osteoporosis and also described in *Ayurveda* for the treatment of osteoporosis as well as bone fracture healing (Sukh Dev., 2006). *L. glutinosa* belongs to the family *Lauraceae* and many of its members are believed to have osteoprotective effect. In the past, the development of herbal anti-osteoporosis formulas was pursued mainly by scientists in Asian countries, including China, Japan and Korea (Xie *et al.*, 2005). Bark of the *L. glutinosa* is used for the preparation of the dried bark powder (Lohita *et al.*, 2010). This bark powder is prescribed directly or used in the formulation for the treatment of osteoporosis. Many herbal formulations which are used for the prevention of osteoporosis are having Maida Lakri as their main herb (Parikh et al., 2009; Sukh Dev, 2006). However, very less scientific data is available about the osteoprotective effect of this plant.

In the present study the osteoprotective property of *L. glutinosa* plant has been explored. This study is the first study describing the osteoprotective effect of *L. glutinosa*.

MATERIALS AND METHODS

Experimental protocol: The experimental protocol was approved by IAEC (Institutional animal ethical committee). Thirty 3-month-old virgin female Wistar rats brought from Sun Pharma Advance Research Center (300±20gm) were used for this study. The animals were acclimatized for 8 days before the onset of the experiment to adapt to laboratory conditions (the room temperature was 22±4 °C with a 12 h/12 h light/dark cycle). Then the rats were ovariectomized (OVX) or sham operated after being anesthetized under intraperitoneal injection of sodium pentabarbitol at a dose of 30mg/kg body weight, as described previously (Xiao *et al.*, 2002). The success of the OVX was assessed through vaginal cytology after five days of surgery as described previously (Roveri *et al.*, 2000). Rats were given the lag phase of 10 days to recover from the stress of operation and experimental animals were divided in 2 main groups, namely control (sham operated) and OVX. They were further categorized and were daily fed with 2% and 5% powder of *L. glutinosa*, using simple RO water as binder (Table 2.1). Every week the feed was checked for any bacterial or fungal infection. If any infection was detected, the entire stock was discarded. Food and water was given *ad libitum.*

Every week blood was collected by orbital sinus puncture. 0.5 ml blood was collected; serum was separated and stored at -80° C for further analysis. At 9^{th} week after the treatment, rats were sacrificed by giving overdose of anesthesia. Rats were dissected, bone, uterus and liver was immediately removed, washed in PBS (pH 7.4), and stored at -80° C for further analysis. Liver and uterus were blotted and weighed. All the assays were carried out using commercial kits purchased from Reckon Diagnostics.

The calcium (Ca) and phosphorus (P) concentrations of serum samples were estimated using standard colorimetric methods and analyzed using automatic analyzer Perkin Elmer as described previously (Beeler and Catrou, 1983; Farrell, 1984; Daly and Ertingshausen, 1972; Gamst *et al.,* 1980). Serum AlP and TRAcP were estimated using pNPP method (George *et al.,* 1975). Serum creatinine profile was analyzed using alkaline picrate method (Kalpan and Pesci, 1984). The histopathological studies were carried for decalcified bone tissue (Fixed in 4% Para-formaldehyde) and uterine tissues (Fixed in 10% neutral formalin). The tissues were dehydrated in an ethanol series and embedded in paraffin and sectioned at 7 µm thickness (bone) and 5um thickness (uterus). The sections were stained with standard Haematoxilin eosin staining.

Statistical Analysis

Data are expressed as mean values and S.E.M. One-way ANOVA was used to compare data from all groups and Bonferroni post test to compare the results ($p < 0.05$ by the statistical software of GraphPad Prism (Version 5.0). A p value of less than 0.05 was considered statistically significant.

RESULTS

Body Weight and Uterine Weight ratio

Rats of both sham as well as the treated OVX group did not show any change in Total Body weight initially. However, at 8^{th} week the OVX group showed a significant increase in the body weight compared to sham group. As shown in Table 2.2, all the rats in all experimental groups had initially similar body weights. Eight weeks after operation, there was significant increase in the body weight of the OVX rats. Along with this, OVX caused significant atrophy of the uterus as anticipated. Compared with the values in the Sham group, the final body weights in the OVX group were significantly increased after 8 weeks (Figure 2.1). There was no significant increase in body weight in the plant treated OVX group. Uterine weight did not show any alteration between OVX and LG treated group (Table 2.3). But when relative uterine weight was taken, it showed a slight increase in the plant treated animals, suggesting the protective effect of this plant (Figure 2.2).

Serum Biochemical Markers

1. **Serum calcium and Phosphate levels**

   The effect of *L. glutinosa* bark powder on serum calcium levels is presented in Figure 2.3. In first two weeks the calcium levels were very fluctuating, but later they stabilized (Table 2.4 and Table 2.5). Ovariectomy appears to reduce serum calcium levels and the plant treatment ameliorates this. However in control plant treatment, it doesn't show any significant change. Ovariectomy also leads to the gradual decrease in serum phosphate levels (Figure 2.4). Plant treatment ameliorates this but data is not significant.

2. **Serum creatinine levels**

   Serum creatinine levels were fluctuating initially but with progression of time they got stabilized and showed no significant difference compared to control (Table 2.6, Figure 2.5).

3. **Serum AlP levels**

   Ovariectomy caused a significant increase in the serum AlP levels, which remained high throughout all 8 weeks (Table 2.7). Plant treatment lowered the serum AlP levels in a dose dependent manner. Control treatment did not show any significant alteration. (Figure 2.6)

4. **TRAcP levels**

   TRAcP levels increased with time in ovariectomized animals in two months (Table 2.8). Plant treatment significantly checked this elevation. Even at 2% dose the TRAcP activity is lowered. At 5% dose it is lowered in the range of control. In control treatment also the plant lowered the TRAcP activity. (Figure 2.7)

Bone and liver AlP levels

Bone AlP levels were significantly higher in the ovariectomized group compared to normal and plant treatment showed lowered AlP levels in a dose dependent manner (Table 2.9, Figure 2.8). In control treatment the bone AlP levels were not significantly altered. Liver AlP levels were lower in ovariectomized animals but the data is not significant (Table 2.10). Plant treatment elevated the liver AlP levels but this data is also not significant (Figure 2.9)

Bone TRAcP levels

Bone TRAcP levels were higher in ovariectomized animals compared to normal. In the plant treated animals, TRAcP levels were reduced, but the reduction was not as significant as serum parameters (Figure 2.10).

Histology:

a. Histology of the uterus

In our study, no significant changes were observed in the uterine epithelium and stroma of control group. The appearance of uterine epithelium and stroma was normal. The covering epithelial cells were a mixture of ciliated and secretory simple columnar cells and the connective tissue of the lamina propria was rich in fibroblasts and contained abundant amorphous ground substance. Connective tissue fibers were mostly reticular (Figure 11a). No significant differences were observed in the control 2% or 5% treatment groups (Figures 11b and 11c). However, the length of the epithelium and the number of the uterine glands decreased, and the appearance of stromal tissue was looser in OVX groups (Figures 12a). Plant treatment did not show any effect on the OVX treated groups treated with 2 % or 5 % plant (Figures 12b and 12c).

b. Histology of bone

Bone histology showed no significant change in all the Sham treatment groups. Histology of the compact and cancellous bone was found to be completely normal in 2% and 5% treatment groups. Bone marrow was also found attached to the cancellous bone (Figures 13a, 13b and13c). However, in the OVX control group, the compact bone did not show any significant alteration, but the cancellous bone was found to be damaged. Histology clearly showed alteration in the bone micro architecture. It was found that in control group intertrabecular bone was maintained well in all three groups. But in ovariectomized animals, trabecular bone was slender and the architecture of bone was lost (Figure 14a). The results were similar to what was observed previously (Kim *et al.,* 2002). In 2 % treatment there was notable improvement in the quality and micro architecture of bone. However, signs of osteoporosis were still seen in the bone (Figure 14b). In 5% plant treatment, the trabecular bone architecture was found to be much preserved and the features were similar to the control group (Figure 14c).

DISCUSSION

The present study demonstrated that feeding the animals with *L. glutinosa* bark powder partially prevents bone loss caused by estrogen deficiency, without affecting the uterus. Ovariectomy of young rat is a model for studying post menopausal osteoporosis (McCann *et al.,* 2005; Kim *et al.,* 2002). As expected, OVX animals in the present study exhibited all the characteristics of weight gain, uterine atrophy, high serum AlP levels etc., which were found to be similar with what was observed previously (Roveri *et al.,* 2000).

Difference in food intake may also affect bone metabolism. Food intake and weight gain were higher in all three OVX groups than in Sham group but were similar between three OVX groups. The effect of *L. glutinosa* bark powder was very different on bone and uterus, which is the primary estrogen target organ. It is widely known that estrogen treatment maintains uterine weight. In contrast, feeding with *L. glutinosa* powder only slightly increased uterine weight. When this data was taken as relative uterine weight, this plant showed ameliorative effect on ovariectomy induced changes. These results were found to be similar with Xie and co-workers (2005).

Serum calcium and phosphate levels decreased in ovariectomized animals, similar with the results described previously (Zhang *et al.,* 2006), which is conflicting with the results presented by Kim *et al.,* (2003) who showed elevation in serum calcium and lowering of serum P. However, in this study, alteration in the serum phosphate ions was statistically not significant. Though plant treatment ameliorated it, data could not be taken into account.

Tartarate resistant ACP is the marker of osteoclastic bone resorption. Main difference seen between TRAcP and AlP was that OVX causes a sudden boost in serum AlP levels, while the TRAcP levels were increasing gradually with time. After ovariectomy there is gradual decrease in the estrogen which is playing a major role in inhibiting the osteoclastic functioning through RANK, RANK L and osteoprotegrin competitive inhibition (Shevde *et al.,* 2000) and that is why the TRAcP levels, which are indication of osteoclastic resorption, were rising gradually with time. However, the bone formation occurs directly in response to resorption, which is seen in the form of elevated AlP levels. However, this bone formation marker shows a little decrease at the end of two months indicating that the osteoblastic cells had lowered their response to resorption. This reduced osteoblastic activity in comparison to osteoclastic activity may result in osteoporosis.

In OVX control, TRAcP increased significantly in two months. *L. glutinosa* bark powder prevents this rise, in a dose dependent manner indicating that this plant is having osteoclast inhibiting property. Ovariectomy causes a significant increase in the serum AlP levels, which remains high for duration of 8 weeks, dropping a little by the end of two months. Plant treatment reduced TRAcP and serum AlP in a dose dependent fashion. This parallel fall in both the markers clearly suggest that this plant ameliorates the damage caused by estrogen deficiency. To confirm that the elevation in serum AlP levels is because of bone remodelling only, we had estimated liver AlP levels also, which show no significant variation between the ovariectomized group and control group.

Uterine histology showed no clear distinction between plant treatment groups indicating that this plant does not have any estrogen potential and must be playing through some other pathway as an osteoprotective agent. Histology of the bone clearly indicated the osteoprotective effect of this plant. The results were similar to that noticed by Kim *et al.,* (2003b) and showed almost protection like that of control in higher dosage group. In the low dose group also it showed osteoprotective effect.

In summary, our results suggest that *L. glutinosa* is having a bone protecting effect without exhibiting substantial effects on the uterus in OVX rats and not having any side effects in the control animals. This plant is having positive effect on bone remodelling and intake of this plant may be useful in preventing bone loss caused by estrogen deficiency.

Table 2.1 Designing of the experimental set up:

Group	Name of the group	No. of animals	Treatment	Abbreviation
A.	Normal control	5	Sham Operated	Sham
B.	Normal treatment	5	Sham Operated + 2% herbal treatment	Sham + 2%
C.	Normal treatment	5	Sham Operated + 5% herbal treatment	Sham + 5%
D.	Ovariectomized control	5	Ovariectomized	OVX
E.	Ovariectomized treatment	5	Ovariectomized + 2% treatment	OVX + 2%
F.	Ovariectomized treatment	5	Ovariectomized + 5% treatment	OVX + 5%

Table 2.2: Body weight.

Week progression	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
1	298.00 ± 4.00	303.00 ± 6.00	301.00 ± 4.00	291.00 ± 6.33	295.00 ± 5.00	288.00 ± 4.00
2	313.00 ± 4.33	312.00 ± 4.67	313.00 ± 3.00	300.00 ± 7.33	301.00 ± 4.67	295.00 ± 4.67
3	328.00 ± 5.00	325.00 ± 4.00	329.00 ± 3.67	325.00 ± 7.00	320.00 ± 4.33	315.00 ± 4.00
4	339.00 ± 4.67	337.00 ± 4.33	335.00 ± 6.33	343.00 ± 5.00	331.00 ± 7.33	330.00 ± 3.67
5	350.00 ± 6.00	350.00 ± 6.33	348.00 ± 4.67	355.00 ± 6.00	350.00 ± 5.00	341.00 ± 3.00
6	355.00 ± 6.33	362.00 ± 4.67	355.00 ± 4.00	376.00* ± 4.33	369.00 ± 6.33	366.00 ± 2.67
7	366.00 ± 5.00	371.00 ± 5.00	368.00 ± 3.67	399.00*** ± 6.33	381.00 ± 4.33	385.00 ± 5.00
8	378.00 ± 3.33	385.00 ± 4.00	381.00 ± 4.33	415.00*** ± 7.33	405.00** ± 7.00	406.00** ± 5.67

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

Table 2.3: Relative uterine weight

	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
Relative Uterine weight	0.001550 ± 0.000167	0.001300 ± 0.000220	0.001559 ± 0.000190	0.000270** ± 0.000183	0.000280** ± 0.000299	0.000270** ± 0.000283

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

Table 2.4: serum calcium profile

Week progression	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
1	9.700 ± 0.030	9.700 ± 0.328	9.700 ± 0.327	9.800 ± 0.298	9.800 ± 0.153	9.800 ± 0.286
2	9.600 ± 0.033	7.960** ± 0.292	8.750 ± 0.286	8.700** ± 0.148	6.700*** ± 0.189	7.400** ± 0.375
3	9.000 ± 0.267	7.800** ± 0.223	7.500** ± 0.222	9.855 ± 0.195	8.600** ± 0.153	7.900*** ± 0.080
4	8.000 ± 0.187	7.900 ± 0.407	7.500 ± 0.174	9.300 ± 0.373	9.458 ± 0.051	8.900 ± 0.186
5	8.400 ± 0.210	9.865** ± 0.195	9.300 ± 0.083	7.600 ± 0.332	9.211*** ± 0.213	9.000*** ± 0.230
6	9.000 ± 0.037	9.300 ± 0.110	9.220 ± 0.333	7.500*** ± 0.189	8.000 ± 0.086	8.100 ± 0.186
7	8.600 ± 0.300	9.300 ± 0.174	9.800*** ± 0.299	6.453*** ± 0.233	7.600** ± 0.293	8.255*** ± 0.233
8	8.800 ± 0.190	8.900 ± 0.153	8.800 ± 0.233	5.458*** ± 0.262	6.600** ± 0.230	8.455*** ± 0.293

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

Table 2.5: Serum phosphate profile

Week progression	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
1	8.300 ± 1.420	7.800 ± 1.303	7.600 ± 1.775	12.600 ± 2.185	12.500 ± 1.101	11.400 ± 0.717
2	7.800 ± 1.690	7.200 ± 1.550	6.200 ± 2.113	12.400 ± 2.600	11.000 ± 1.310	9.900 ± 1.310
3	7.600 ± 1.780	6.300 ± 1.633	5.200 ± 2.225	14.600 ± 2.738	9.100 ± 1.380	9.600 ± 1.380
4	6.800 ± 2.350	5.200 ± 2.156	5.300 ± 2.938	16.500 ± 3.615	9.600^{**} ± 1.822	8.500^{**} ± 1.822
5	7.800 ± 1.230	5.400 ± 1.128	5.400 ± 1.538	16.800^{**} ± 1.892	9.400^{**} ± 0.953	7.300^{***} ± 0.953
6	7.600 ± 1.890	5.700 ± 1.734	5.600 ± 2.363	17.100 ± 2.908	9.200^{**} ± 1.465	7.600^{***} ± 1.465
7	7.600 ± 1.540	5.800 ± 1.413	5.700 ± 1.925	17.900 ± 2.369	8.900^{***} ± 1.194	7.900^{***} ± 1.194
8	7.900 ± 1.230	5.900 ± 1.128	5.800 ± 1.538	18.200^{**} ± 1.892	8.800^{***} ± 0.953	7.500^{***} ± 0.953

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

Table 2.6: serum Creatinine profile.

Week progression	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
1	0.820 ± 0.100	0.790 ± 0.080	0.714 ± 0.090	0.956* ± 0.080	0.760 ± 0.050	0.720 ± 0.12
2	0.854 ± 0.090	0.759 ± 0.090	0.580 ± 0.050	0.912 ± 0.110	0.691 ± 0.110	0.7120 ± 0.11
3	0.799 ± 0.080	0.754 ± 0.110	0.714 ± 0.060	0.854 ± 0.040	0.631 ± 0.090	0.744 ± 0.07
4	0.734 ± 0.120	0.929 ± 0.120	0.785 ± 0.040	0.774 ± 0.080	0.731 ± 0.050	0.794 ± 0.05
5	0.785 ± 0.090	0.831 ± 0.100	0.781 ± 0.110	0.735 ± 0.020	0.722 ± 0.047	0.855 ± 0.09
6	0.799 ± 0.080	0.845 ± 0.040	0.745 ± 0.060	0.841 ± 0.070	0.764 ± 0.010	0.814 ± 0.08
7	0.789 ± 0.110	0.821 ± 0.050	0.729 ± 0.110	0.754 ± 0.050	0.822 ± 0.090	0.735 ± 0.06
8	0.813 ± 0.130	0.847 ± 0.080	0.889 ± 0.090	0.789 ± 0.080	0.804 ± 0.060	0.8874 ± 0.11

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

Table 2.7: serum AlP profile

Week progression	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
1	57.21 ± 1.66	50.33 ± 2.66	52.12 ± 3.00	157.00^{***} ± 2.66	153.00 ± 1.66	155.00 ± 4.00
2	58.20 ± 3.33	54.12 ± 3.00	56.37 ± 1.66	152.32^{***} ± 3.66	145.86 ± 3.66	134.25^{***} ± 3.66
3	57.24 ± 2.66	51.23 ± 3.66	57.56 ± 2.00	152.64^{***} ± 1.33	149.36 ± 3.00	130.69^{***} ± 2.33
4	52.80 ± 1.66	50.87 ± 4.00	54.92 ± 1.33	153.21^{***} ± 2.66	136.17^{***} ± 1.66	110.69^{***} ± 1.66
5	49.68 ± 2.00	55.54 ± 3.33	45.56 ± 3.66	143.86^{***} ± 3.00	134.53 ± 1.33	106.52^{***} ± 3.00
6	54.42 ± 3.00	51.12 ± 1.33	42.98^{*} ± 2.00	139.08^{***} ± 2.33	127.91^{*} ± 3.66	94.18^{***} ± 2.66
7	53.21 ± 3.66	44.54 ± 1.66	40.85^{*} ± 3.66	128.96^{***} ± 1.66	117.48^{*} ± 3.00	87.52^{***} ± 2.00
8	53.79 ± 2.66	54.14 ± 2.66	40.95^{**} ± 3.00	133.99^{***} ± 2.66	118.28^{***} ± 2.00	79.280^{***} ± 3.66

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

Table 2.8: Serum TRAcP profile

Week progression	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
1	8.30 ± 0.18	7.80 ± 0.29	7.60 ± 0.30	12.60*** ± 0.89	12.50 ± 0.18	11.40 ± 0.32
2	7.80 ± 0.04	7.20 ± 0.30	6.20 ± 0.17	12.40*** ± 0.90	11.00 ± 0.29	9.90*** ± 0.28
3	7.60 ± 0.29	6.30 ± 0.03	5.20** ± 0.21	14.60*** ± 0.91	9.10*** ± 0.15	9.60*** ± 0.24
4	6.80 ± 0.17	5.20** ± 0.04	5.30 ± 0.14	16.50*** ± 1.24	9.60*** ± 0.26	8.50*** ± 0.28
5	7.80 ± 0.21	5.40* ± 0.06	5.40** ± 0.03	16.80*** ± 1.10	9.40*** ± 0.04	7.30*** ± 0.15
6	7.60 ± 0.30	5.70* ± 0.15	5.60* ± 0.21	17.10*** ± 0.99	9.20*** ± 0.32	7.60*** ± 0.26
7	7.60 ± 0.03	5.80* ± 0.17	5.70* ± 0.07	17.90*** ± 0.98	8.90*** ± 0.28	7.90*** ± 0.32
8	7.90 ± 0.27	5.90* ± 0.17	5.80** ± 0.30	18.20*** ± 0.78	8.80*** ± 0.14	7.50*** ± 0.24

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

Table 2.9: Bone AlP levels

	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
Bone AlP Levels	337.00 ± 7.120	351.000 ± 11.600	355.000 ± 10.600	555.480^{***} ± 18.300	481.280^{***} ± 14.120	393.900^{***} ± 10.600

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

Table 2.10: Liver AlP levels

	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
Liver AlP Levels	44.760 ± 7.000	56.480 ± 11.650	39.810 ± 10.600	36.890 ± 11.250	44.220 ± 14.000	57.666 ± 10.253

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

Table 2.11: Bone TRAcP levels

	Control	Control + 2%	Control + 5%	OVX	OVX + 2%	OVX + 5%
Bone TRAcP Levels	5.090 ± 0.162	4.859 ± 0.290	4.565 ± 0.262	12.126^{***} ± 0.329	11.560 ± 0.300	9.804^{**} ± 0.262

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

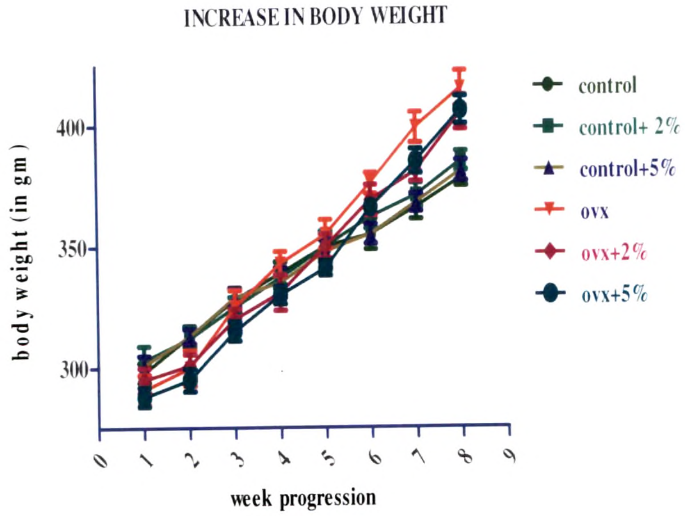

Figure 2.1 Showing graphical representation of increased body weight

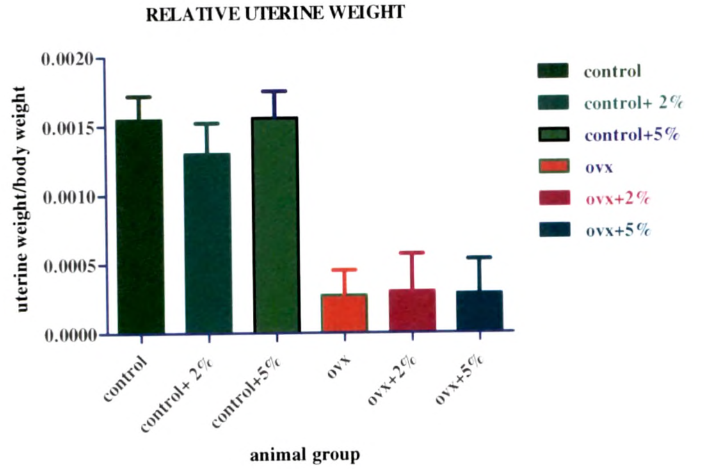

Figure 2.2 Showing graphical representation of changes in uterine weight

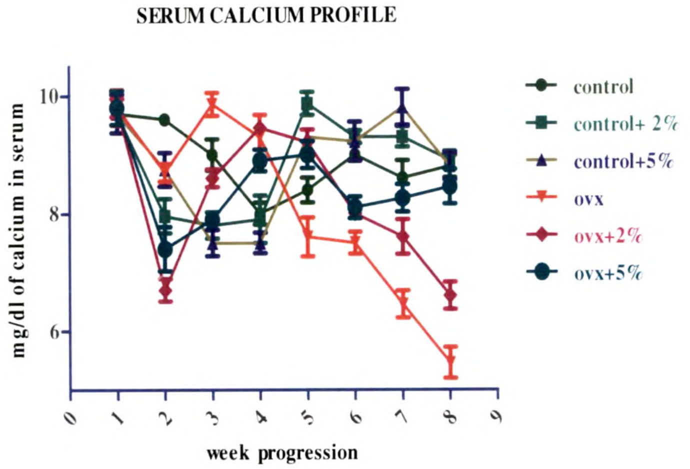

Figure 2.3 Showing graphical representation of alteration in serum calcium profile

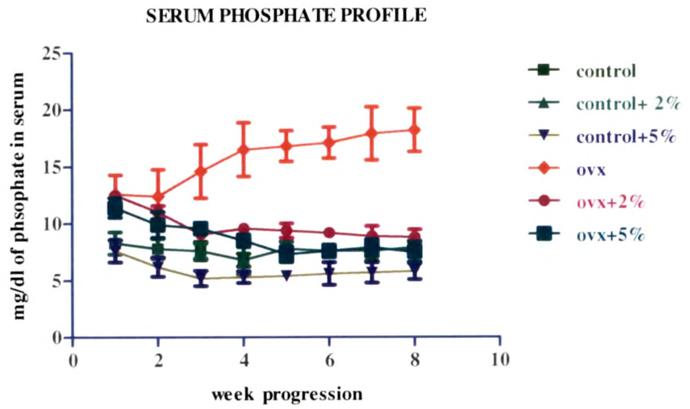

Figure 2.4 Showing graphical representation of alteration in serum phosphate profile

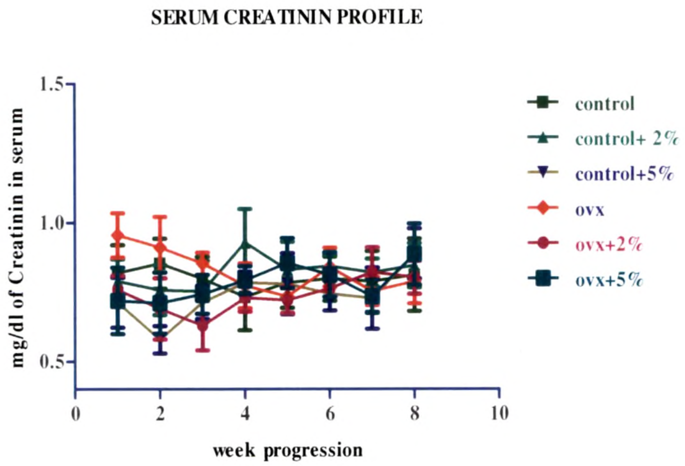

Figure 2.5 Showing graphical representation of alteration in serum creatinine profile

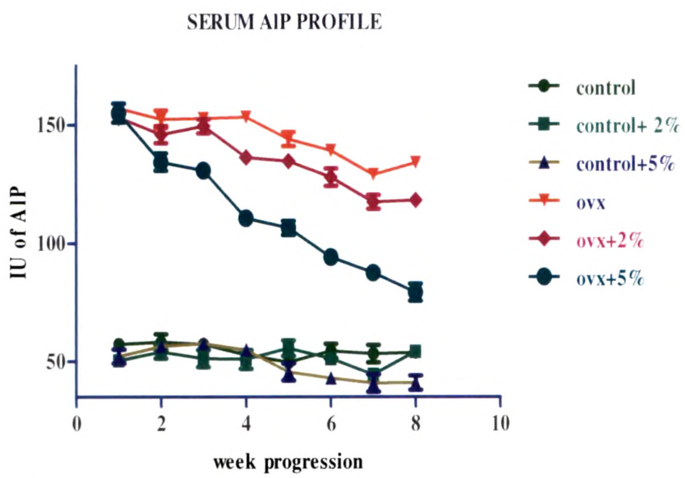

Figure 2.6 Showing graphical representation of alteration in serum AlP profile

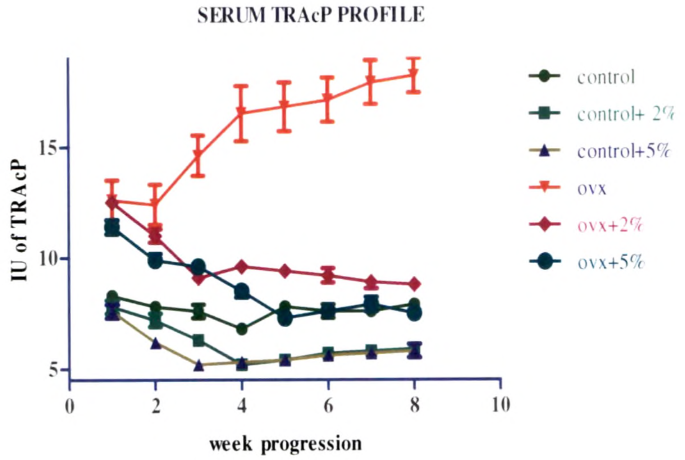

Figure 2.7 Alterations in Bone tissue TRAcP levels with LG treatment

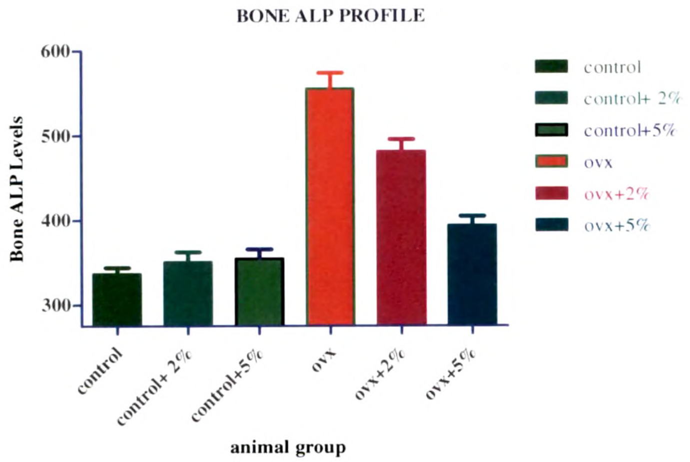

Figure 2.8 Alterations in Bone tissue ALP levels with LG treatment

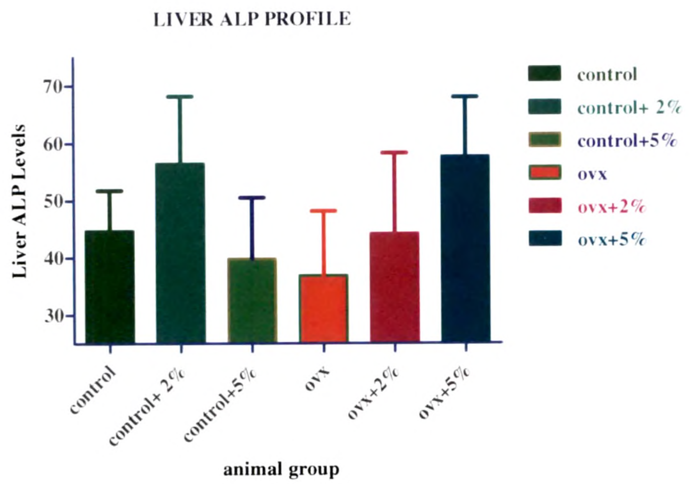

Figure 2.9 Showing graphical representation of alteration in Liver tissue AlP profile

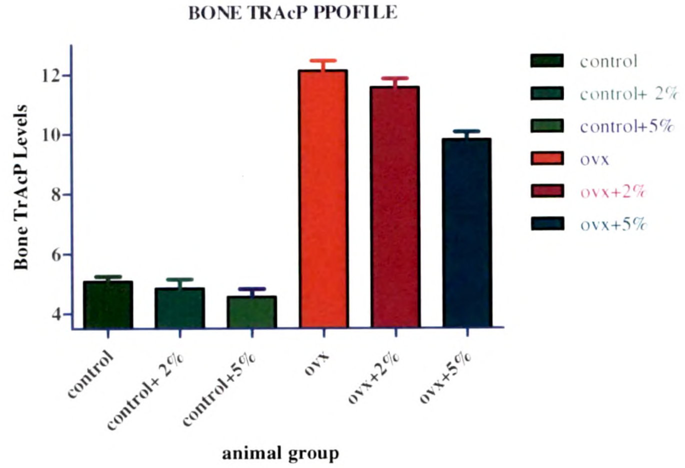

Figure 2.10 Showing graphical representation of alteration in Bone tissue TRAcP profile

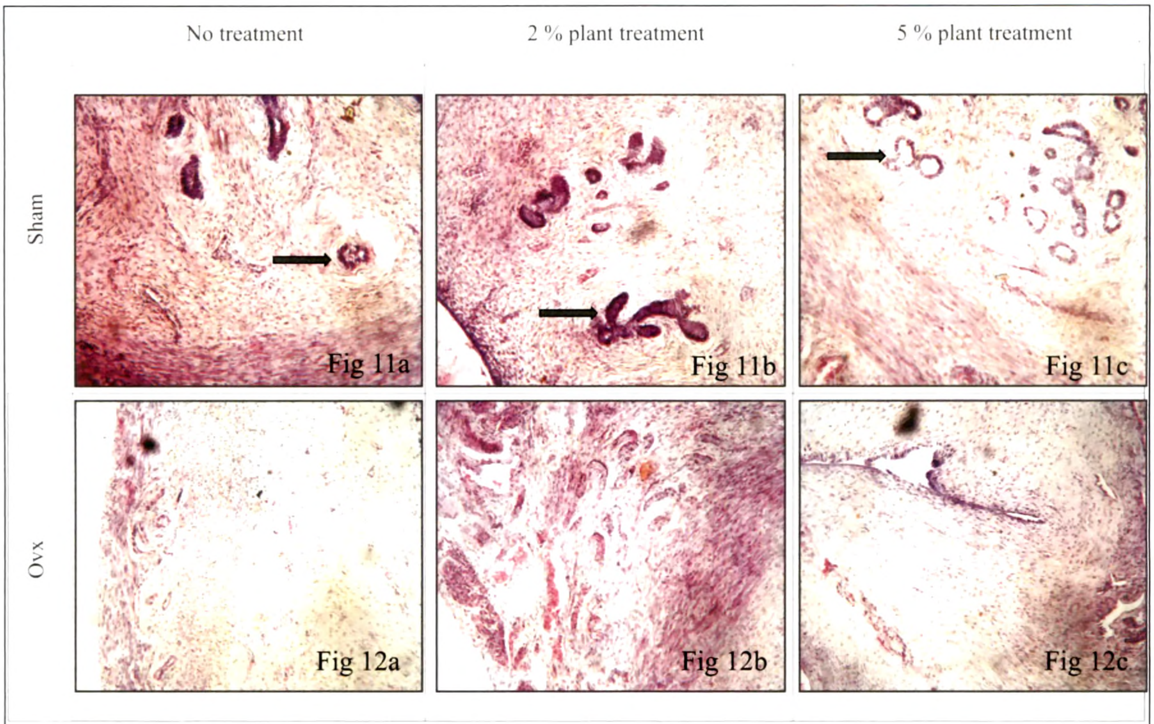

Figure 11 uterine histology of Sham groups; presence of uterine glands(→)was well marked
Figure 12 uterine histology of OVX groups; indicating overall reduction in growth of tissue

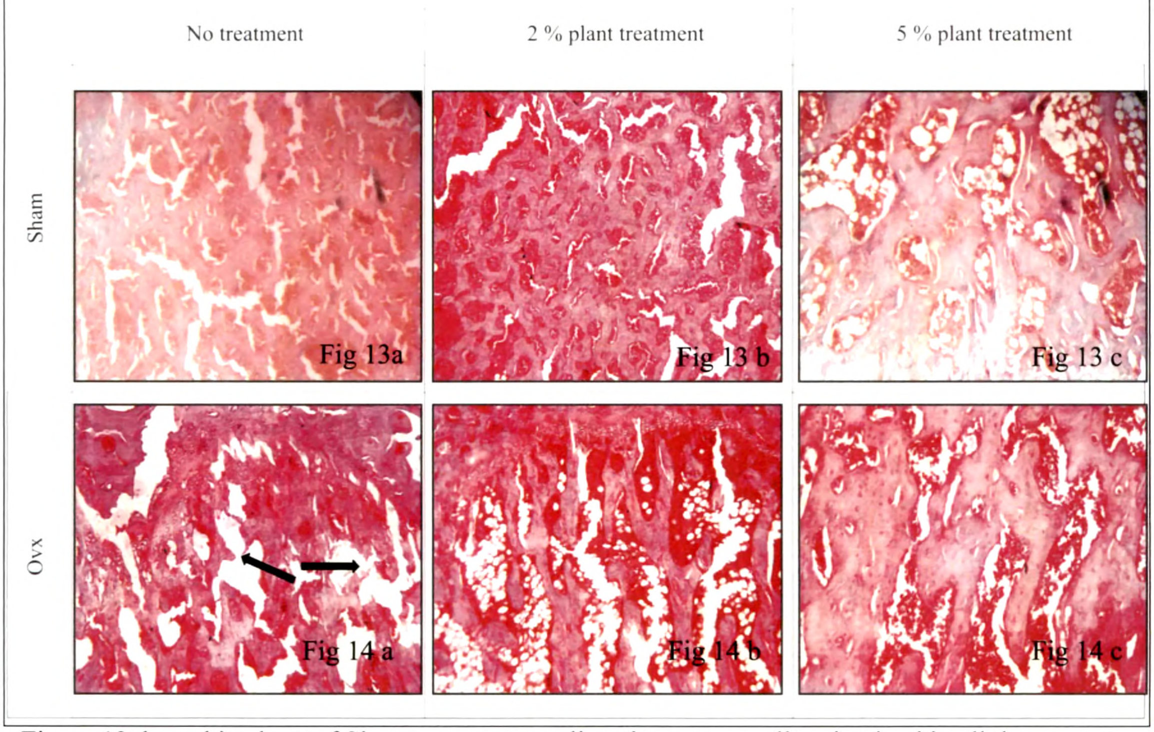

Figure 13 bone histology of Sham groups; cancellous bone was well maintained in all three groups
Figure 14 bone histology of OVX groups; cancellous bone was damaged (→)in OVX control, LG treatment improved the quality of bone in a dose dependent manner