CHAPTER 3

EFFECCT OF LG METHANOLIC EXTRACT ON BONE MARKERS, CALCIUM METABOLISM AND BONE HISTOARCHITECTURE

Chapters 1 and 2 have established osteoprotective effect of *LG*. The aim of the present study was to evaluate the efficacy of *LG* methanolic extract in preventing bone loss ovariectomized rats by evaluating its effects on calcium metabolism, various biochemical markers and bone histomorphometry.

INTRODUCTION

Osteoporosis that is related with ovarian hormone insufficiency following menopause is by far the most common cause of age related bone loss (Reddy and Lakshamana, 2003). Rapid decline in the levels of estrogen immediately after menopause is decisive to the pathogenesis of osteoporosis (Riggs and Melton, 1995; Schmidt *et al.,* 2000). Rapid and continuous bone loss occurs for several years after menopause due to absence of Estrogen. This accelerated bone remodelling at menopause is mainly due to the increased levels of both bone resorption (up to 150%) and bone formation (up to 100%); leading to an imbalance in bone remodelling. Increased bone resorption is associated with faster bone loss and deterioration of bone microarchitecture, both in the trabecular compartment (trabecular perforations, loss of entire trabeculae, deterioration of the trabecular connectivity) and in the cortical compartment (cortical thinning, increased cortical porosity). However, this bone loss is dependent upon various factors, including ethnicity and race, as they are well known determinants of skeletal health and bone mineral density. A recent study reports 29% prevalence of osteoporosis in 30-60 year age group Indian women. BMD values in Indian population are approximately 15% lower than those in Caucasian women. In addition, fractures are reported to occur 10 – 20 years earlier in Indians when compared with the western population. One of the reasons could be that majority of Indians have low vitamin D status and are on low dietary calcium which makes them prone to bone diseases (Reddy and Lakshamana, 2003).

Estrogen replacement therapy is approved for the prevention of bone loss in postmenopausal women and is efficacious in reducing the incidence of skeletal fractures (Turner *et al.,* 1994). However, estrogen use and compliance are limited due to its numerous undesirable side effects such as uterine and breast cancer (Lindsay and Cosman, 1992). Unfortunately there are few therapies available to treat or prevent osteoporosis and aid bone healing apart from the bisphosphonates (e.g. risedronate), HRT (hormone replacement therapy) with natural and synthetic oestrogens, and calcium plus vitamin D, all of which have significant drawbacks (Hidaka *et al.,* 2006). Hence, it would be most helpful to explore naturally occurring substances especially of plant origin that could prevent bone loss and free from any adverse effects. Many consumers would also prefer to avoid synthetic molecules in favour of natural products (Reddy and Lakshamana, 2003).

The OVX model in rat has been particularly useful to study the efficacy of various agents for the prevention and for reversal of bone loss (Kalu *et al.,* 1989). It has also been shown to exhibit physiological condition similar to what is observed in post menopause. It also exhibits biochemical markers of bone resorption which have been used to illustrate metabolic bone diseases and gauge therapeutic response in postmenopausal women. The amount of bone and resorption rate at a specific time point can be determined by measuring the levels of various biochemical markers of bone. Markers provide dynamic information that is not possible with a single measurement of BMD or bone histomorphometry. Bone turnover markers are very useful in the identification, selection of anti-osteoporosis treatment, i.e. Serum TRAcP reflects the number and activity of osteoclasts on bone surface, while serum AlP reflects bone formation. Fall in serum calcium and hydroxyproline levels and increased elimination of calcium in urine reflects resorption process (McDonald *et al.,* 2004). Generally serum markers exhibit less total intra-individual variability than urine markers, thus to develop more clear picture, metabolic studies are undertaken which allows to study the complete metabolism of bone elements (Roveri *et al.,* 2000).

Ayurveda, an ancient system of Indian medicine mentions several plants that are useful in the correction of bone metabolic disorders such as osteoporosis (Reddy and Lakshamana, 2003). *LG* (Lour.) C.B.Rob., *Lauraceae* is moderately-sized tree; bark thin, grey or pale brown; live bark 3 mm thick, pale brown, very slimy. It contains flavone glycosides, reducing sugars, amino acids and tannins and has been reported to have anti oxidant as well as wound healing activity (Devi and Meera, 2010). In the same study, this plant was reported to have anti inflammatory activity, and it has been reported that plant with anti inflammatory activity are potent candidates for osteoprotective effect. Recently, research disclosed that the MeOH extract of *LG* bark effectively inhibited both Gram-positive and Gram-negative bacteria, justifying its use in diarrhoea and dysentery (Mandal *et al.,* 2000). In our previous study it had been observed that this plant is having osteoprotective effect, but its role in ameliorating biochemical markers was not clear. Thus it was thought pertinent to assess the role of this plant in bone metabolism in long term study and to develop a clearer picture about its pharmacological use as an osteoprotective agent.

The main objectives of the proposed study are to evaluate the efficacy of *LG* methanolic extract for its usefulness in preventing bone loss ovariectomized rats by evaluating its effects on calcium metabolism, various biochemical markers and bone histomorphometry.

MATERIALS AND METHODS

Preparation methanolic extract of *LG*

The dried stem bark powder was acquired from local drug market and was extracted with methanol (50–60° C), using soxhlet's apparatus for 48 hours. The extract was collected and dried on water bath to yield a semi solid paste, which was then freeze dried to yield solid extract. This extract was suspended in 0.05% carboxy methyl cellulose before oral gauging.

Protocol

All the protocols for animals' treatment and utility were approved by Institutional animal ethical committee before procuring the animals. Forty two, 3 month old female, virgin Wistar rats weighing between 225 and 250 g were obtained from Sun Pharma Advance Research Centre and maintained in standard laboratory conditions. After 2 weeks of adaptation, they were randomized by weight into seven groups of 6 each. Group 0 or absolute control animals were not given any treatment or exposed to any surgery. Group 1, 2 and 3 rats were sham operated while group 4, 5 and 6 were bilaterally ovariectomized. All the animals were fed with commercial palleted diet (Pranav Agrofood) containing calcium and drinking water *ad libitum*. Ovariectomy was confirmed by observing the vaginal cytology after 5 days of operation. The animals were allowed a week to recover and then treatment was started as follows:

Group 0: Normal control.

Group 1: Sham control and received vehicle.

Group 2: Sham control and received 200mg/kgbw plant extract daily.

Group 3: Sham control and received 400mg/kgbw plant extract daily.

Group 4: Ovariectomized control and received vehicle.

Group 5: Ovariectomized and treated with 200 mg/kgbw plant extract daily.

Group 6: Ovariectomized and treated with 400 mg/kgbw plant extract daily.

Treatment was continued for 12 weeks maintaining 12 h/ 12 h light/dark illumination cycles at constant temperature (24 ± 2 °C) and 40 – 50 % humidity. Urine excreted over 24 h period was collected from overnight fasted rats by housing each rat individually in metabolic cages. Fasting blood samples were collected on ice from the study and control groups' every10th day for estimating biochemical markers of bone turnover. Animals were housed in metabolic cages and total urine and faeces were collected in a sterile calcium free container for the determination of urine levels of calcium and hydroxyproline, as well as feaces collection. At the end of the study,

animals were killed by over dose of anesthesia and blood collected was centrifuged to separate serum and preserved in -70°C until assay. Rats were dissected, bone, uterus and liver was immediately removed, washed in PBS (pH 7.4), and stored at -80° C until analysis. Liver and uterus were blotted and weighed. All the assays were carried out using commercial kits purchased from Recon Diagnostics.

Serum and urine analysis

Serum levels of calcium, hydroxyproline, total alkaline phosphatase (AlP), TRAcP, urinary calcium and hyrdoxy proline were measured using Perkin Elmer autoanalyser. Serum concentrations of Calcium, TRAcP and AlP were estimated by commercial kits purchased from Reckon Diagnostics Ltd. (Vadodara) Serum and urinary Hydroxy proline levels were measured using the method of Neumann and Logan (1950) modified by Levine (1972).

Feed and faecal calcium and hydroxyproline analysis.

For calcium and hydroxyproline content of the faeces and food, they were homogenised in 6 N HCl and extracted in the same for 24 hours as described previously (Roveri *et al.*, 2000). After 24 hours, the solution was filtered, concentrated on sand bath to yield a white crystalline powder. This powder is then dissolved in Tris buffer pH 8.8 and analyzed using commercially available test kit for calcium.

Calcium Balance Study

Faeces were dried at 120 °C and milled; the powdered faeces was then dissolved in 6 M HCl, warmed to 80 °C for 10 min. Faecal calcium content was measured by a colorimetric method using a kit similar to that for analyzing serum samples. Calcium intake was estimated from daily food intake. The absorption and retention rates of calcium were calculated by the following equations:

Ca intake = (calcium in food X daily consumption of food) + (calcium in water X daily consumption of water)

Ca absorption rate (%) = (Ca intake - faecal Ca)/Ca intakeX100;

Ca balance = Ca intake - (urinary Ca + faecal Ca);

Ca retention rate (%) = Ca balance/Ca intakeX100.

Bone Calcium and collagen Content:

For estimation of bone calcium, right tibia was removed immediately after surgery and freed of adherent tissues. Bone was allowed to get dissolved in 6N HCl for 48 hours. After complete

dissolution, the solution was evaporated on sand bath to yield white crystalline powder. This powder was dissolved in Tris buffer pH 8.8 and analyzed using commercially available test for calcium and Levin's (1972) modified method for hydroxyproline.

Histology

For histology of bone, fresh bones were fixed in 4% buffered paraformaldehyde for 24 hours. After fixation bones were washed and decalcified in 10% formic acid for 24 hours.

Decalcified bones were collected and outsourced for preparation of wax blocks and Haemetoxilin eosin staining using standard method.

Scanning electron microscopy

The right femurs were fixed in 4% paraformaldehyde solution for 24 hours. The bones were then dehydrated in ethanol, dried, mounted on stubs, coated with gold and observed on a JEOL JSM 300 microscope (Ayaz *et al.,* 2009).

Statistics

All statistical analysis was done by using one way analysis of variance (ANOVA) followed by Bonferroni post hoc test to determine any significant difference between the groups using Graphpad Prism 5 software. Differences between means at the 5% confidence level ($P<0.05$) were considered to be statistically significant as described previously (Parikh *et al.,* 2009).

RESULT

Food consumption and body weight:

As shown in the table 3.1, the mean weekly food consumption of all the Sham animals was low after operation and started increasing from the first week and reached a plateau at the 2nd week after surgery. However, OVX animals showed increase in food consumption till 5th week and it got steady after that (Figure 3.1). Initial low food consumption in both the animals was indication of the stress of operation, as in group 0 there was no significant alteration in the food consumption throughout the study period. There was no significant variation between the weight gain of the Normal control and Sham operated animals. However, there was significant weight gain in the OVX animals (Table 3.2). Weight gain was higher in OVX rats compared to sham groups, and *Litsea* treatment prevented weight gain in both sham and OVX animals (Figure 3.2).

Uterine atrophy

We observed a decrease in the uterine weight of all OVX animals as shown in table 3.3. There was no significant difference observed between Sham operated and control animals. OVX animals showed a significant decrease in the uterine weight, indicative of lack of Estrogen. *Litsea* treatment did not induce any change in the uterine weight in sham or OVX animals. However, when the relative uterine weight was taken, it was seen that this plant is having some effect on the plant treated animals (Figure 3.3).

Serum and urine analysis of calcium

The effect of *LG* on serum and urinary calcium is shown in Table 3.4 and 3.5 respectively. The results indicate a significant reduction ($P<0.05$) in the serum calcium in OVX rats when compared with sham and *Litsea* treated animals (Table 3.4, Figure 3.4). Results clearly showed that there is gradual fall in the serum calcium levels, while the urinary calcium levels are rising. It was observed that OVX animals have more urinary excretion of calcium (table 3.5). Treatment of *LG* lowered the calcium loss in urine in a dose dependent manner (Figure 3.5). However, the significance of this data is not continuous, but when we take this as excretory rate by taking the ratio of serum and urinary calcium levels (Table 3.6), we can see a continuous excretory rate in sham operated animals of group 1. In group 2 and 3, we do not see any observable changes in the excretory rate. Group 4 animals, which were ovariectomized, showed significantly increased excretory rate compared to Sham group and it also showed increase in excretory rate with progression of time (Figure 3.6). *L. glutinosa* treatment significantly reduced this excretory rate

by ameliorating both the parameters, as it reduced urinary calcium levels and improved serum calcium fall. The plant was found to be very potent in ameliorating the increase in excretory rate.

Serum and urine analysis of hydroxyproline.

Hydroxyproline is one of the important amino acid found in collagen, structural constituent of the bone matrix. Similar pattern with lower significance was observed in hydroxyproline. Results showed increased hydroxyproline excretory rate in OVX groups when compared with sham groups. In control animals it had no significant effect, while in group 5 and 6; *Litsea* treatment checked the hydroxyproline excretory rate in a dose dependent manner (Table 3.7, Figure 3.7).

Serum AlP

Table 3.8 shows the increased AlP levels in all the OVX groups. Our study showed no difference between control and Sham operated groups. However, it showed clear significant elevation of AlP indicating osteoporosis induction in OVX animals. In group 5 *Litsea* treatment lowered the AlP levels to a certain extent. In high dose group 6, further reduction was seen. However, this reduction was not observed in a dose dependent manner, contrasting to other results (Figure 3.8).

Serum TRAcP

Table 3.9 shows the increased TRAcP levels in all the OVX groups compared to sham groups. *LG* significantly checked the elevation of the serum TRAcP in a dose dependent manner, brining the TRAcP levels almost to Normal in high dose group (Figure 3.9). It was also notable that in sham groups also, *LG* treatment lowered the TRAcP levels, however the reduction was statistically non significant in sham groups.

Calcium metabolism

A total account of daily intake and excretion of the calcium was monitored to see the effect of Ovariectomy and *LG* treatment on calcium metabolism. There was no significant difference seen between calcium intake of the animals; OVX animals showed higher calcium intake, which was directly dependent upon higher food intake (Table 3.10). When it was taken as Ca absorption rate, there was no significant difference observed between the groups, indicating that this plant is not having any role in increasing or decreasing the calcium absorption through alimentary canal (Table 3.11). It appears from the absorption rate of calcium that there is no alteration in the Calcium metabolism. However, when it was taken as Ca retention rate, it was observed that Ovariectomy leads to alteration in calcium retention rate (Table 3.12). LG tries to ameliorate the changes induced by Ovariectomy. However, the data was not observed in a dose dependent

manner and LG showed very weak effect on altered calcium metabolism. Though, the food intake, and thus calcium intake of the animals is more, there is elevated excretion of calcium, both in urine and feaces. We can see that, though food intake is reduced in *Litsea* treated animals, their, urinary excretion of calcium is low and the fecal excretion of the calcium is also reduced. When entire data was taken collectively as calcium retention rate, OVX animals showed a marked reduction in calcium retention while *LG* treatment shows a prominent effect on the calcium retention rate in OVX animals. The changes observed in the calcium metabolism were not as prominent as the excretory rate (Figure 3.10).

Bone Calcium content

As shown in the table 3.13, bone calcium content was significantly reduced in OVX animals, indicative of osteoporosis. There was no difference observed between the Normal control and any of the sham groups. However, *LG* treatment improved the bone calcium content. Bone calcium content was found to be higher in 400mg plant extract treated animals, however, it was still lower compared to sham group (Figure 3.11).

Histology of bone: In order to study the effects of *LG* on the bone of ovariectomized rats, we carried out histology to study the histo-architecture of bone. It was found that in control group the connection of the intertrabecular bone was well maintained (Figure 3.12), but in OVX group, trabecular bone was slender and the connection of intertrabecular bone was lost (Figure 3.13). *Litsea* treatment had significant effect on this ovariectomy induced damage and it prevented the bone loss in a dose dependent manner. In 200 mg/kg bw treatment, the trabecular bone was improved compared to OVX group and the intertrabecular connections were found to be less damaged (Figure 3.14). In 400 mg/kg bw treatment, histo architecture showed significant improvement, as the intertrabecular bone was found to be reverting to that of sham group and showing improved intertrabecular connections (Figure 3.15).

Scanning electron microscopy

We utilized the SEM technique to determine the pattern of bone resorption at the epiphyseal region of distal femur, which predominantly contains the areas on bone resorption (Figure 3.12 as control). Porous and erosive appearance of femur at the epiphyseal edges was more pronounced and prevalent in OVX animals when compared with sham operated (Figure 3.13). The scanning electron photomicrographs clearly indicate an extensive resorption in ovariectomized animals when compared with sham operated animals. The results were found similar to histology, as there

was no significant alteration when compared with the sham groups. Treatment with *Litsea* decreased the resorption of minerals and maintained the intactness and integrity of the surface indicating its usefulness in the prevention of bone loss. Similarly, *Litsea* treatment showed a clear osteoprotective effect in group 5 and 6 in a dose dependent manner (Figure 3.14 and Figure 3.15). Our findings were found to be similar with what was observed by Matthew *et al.,* (2004).

DISCUSSION

It is well known that both Estrogen and calcium deficiencies are important risk factors in the pathogenesis of osteoporosis. These changes are partly due to hyperparathyroidism secondary to calcium deficiency and exacerbated by estrogen deficiency (Riggs and Melton, 1986). *LG* had been found to have beneficial effect on bone. The present study was undertaken to investigate its usefulness, efficacy and probable pathway of its pharmacological role in treating osteoporosis.

This study clearly demonstrates the usefulness and beneficial effects of *LG* in the prevention of bone loss induced by ovariectomy and prevented calcium loss. Our data on body weight, uterine weight, bone calcium content and histoarchitecture, serum profile and calcium metabolism of OVX animals confirmed the observation of other investigators (Minkin, 1982; Hidaka *et al.,* 1997; Hidaka *et al.,* 2003). We observed that OVX animals had gained more weight compared to control animals and their food intake was also higher. The increase in food consumption by OVX rats may in part contribute to the increase in body weight as observed previously (Hidaka *et al.,* 1997; Hidaka *et al.,* 2003). Reduction in the body weight with *LG* is probably due to less food consumption and can be due to presence of thiocoumarins. Our observations are in agreement with Carlton and co workers (1996) who have reported effect of coumarin in Sprague-Dawley rats and CD-1 mice.

Fasting urinary calcium excretion could also be used as an important variable for estimating net bone resorption. Nyda and co workers (1948) were the first one to observe that ovariectomy leads to decrease in serum Calcium and increased urinary excretion of calcium. Our results indicated similar profile of serum and urinary calcium. We also observed that excretory rate of calcium was reduced with *Litsea* plant treatment in a dose dependent manner. In low dose group also the plant treatment was able to encounter the rise in excretory rate. In 400mg/kgbw dose, the condition was almost similar to the sham groups, suggesting potent osteoprotective role of this plant by checking the increase in the excretory rate.

A marked atrophy of the uterus has been used as evidence of the success of the ovariectomy, because estrogen directly influences uterine weight (Hidaka *et al.,* 1997; Hidaka *et al.,* 2006). Since, *Litsea* treatment did not induce any change in the uterine weight in sham or OVX animals, it is suggested that these agents do not have any influence on the actions of estrogen or its receptors (Clark *et al.,* 1982). This data was found to be highly significant when relative uterine weight was taken, indicating uterine atrophy and protective role of this plant in Estrogen deficiency. Our previous studies had also confirmed these changes that this plant is not having any significant estrogenic effect on the uterus (Parikh *et al.,* 2009). Ishida *et al.,* (1998) had reported that OVX animals had uterine atrophy and it could be prevented by estrogen but not with the phytoestrogens like genistin or diadizine.

Apart from body weight, OVX rats developed similar changes to those seen in postmenopausal women as indicated by increased serum AlP and gradually increasing TRAcP. Serum TRAcP level is one of the marker of increased osteolysis (Lee *et al.,* 2004) while Serum AlP is an important biochemical marker of bone formation and increased osteogenesis (Kim *et al.,* 2003). Ohta *et al.,* have reported increase in the concentration of serum AlP is due to the increase in osteogenesis in response to the amplified osteoclastic resorption. Treatment with *Litsea* restored the increased levels of serum AlP indicating its usefulness in osteoporosis. The level of this enzyme is increased in osteoporosis and other bone metabolic disorders (Victor, 1993). One important phenomenon observed was that AlP levels remains high throughout the study period, while the TRAcP levels were gradually rising, suggesting that osteoclastic activity is increasing slowly, while the osteoblastic activity is remaining steady at an increased level. At the end of three months, we observed that AlP levels are gradually reducing, while TRAcP levels still remained increasing, indicating that now the response of osteogenesis to resorption is decreased and osteoblasts are no more able to keep up with the increased osteoclastic resorption. *LG* significantly checked the TRAcP activity, indicating its effect on Osteoclastic resorption. And as the osteoclastic activity is checked, osteogenesis, which is linked to resorption, is also reduced, which was observable in the form of reduced AlP activity.

As calcium metabolism is getting altered during osteoporosis, animals were taken in to study for their calcium metabolism. We observed that LG had no significant effect on the calcium absorption rate, indicating that this plant is not having any effect on the absorption of calcium in the intestine, and it is playing role as an osteoprotective agent by directly reducing the excretory

rate from urine. This plant also showed a weak effect as an osteoprotective agent in calcium retention rate, suggesting that this plant is mainly functioning through directly acting upon main calcium pool - bone, rather than affecting other organs that carry out mineral homeostasis. This assumption was supported by bone calcium content, as we could observe that calcium content of the bones was improved in a dose dependent manner with *Litsea* treatment. Lee *et al.,* (2004) also observed similar results and showed that bone calcium content is one of the important parameter to assess the status of bone mineralization.

The distal metaphyseal region of femur that contains both cortical and trabecular bone is very sensitive to estrogen deprivation and results in rapid and profound osteopenia (Westerlind *et al.,* 1997). Previous publications have described the need to study both cancellous and cortical bone resorption for osteoporosis agents (Mazess, 1990), however, bone loss usually does not occur at the periosteal surface (Higdon *et al.,* 2001). This phenomenon was clearly depicted in our study as bone histology showed no significant change in sham groups but resulted in severe damage in OVX animals. Compact and cancellous bone was found to be completely normal in sham groups. Bone marrow was also found attached to the cancellous bone. Contrary to this, OVX control group, compact bone did not show any significant alteration, but cancellous bone showed altered bone micro architecture. Intertrabecular bone structure was well maintained in all control groups. In OVX animals however, trabecular bone was seen to be thinning and the quantity of bone was lost. *Litsea* treatment showed a notable improvement in the quality and micro architecture of bone. It is known that histology and scanning electron microscopy (SEM) are the techniques employed for studying the osteoporosis in bones (Ohta *et al.,* 1992). Though, SEM is being used extensively in understanding the elemental composition of metal and alloys, it is quite a useful tool in studying the architecture of biological structures. Our study supported the results observed by Ohta *et al.,* (1992a) and show thinning of bone trabeculae and increase in the pore size of the bones. Litsea treatment improved the bone microarchitecture in a dose dependent manner, indicating that this plant is a potent osteoprotective agent.

In conclusion, *Litsea* treatment in an adult rat model of post menopausal bone loss had shown promising and beneficial effects on the excretory rate of calcium and maintained the calcium metabolism. It also ameliorated that changes induced bone histoarchitecture due to ovariectomy, suggesting that consumption of this plant ingredients can help in preventing osteoporosis.

Table 3.1: Weekly Average Food Consumption:

Week	Normal Control	Control	Control + 200 mg	Control + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	13	9.9	8.9	9.6	10.2	10.1	9.7
2	13	11.7	13.2	12.9	12.7	12.7	11.7
3	12	13	13.2	13.7	13.8	13	14.3
4	14	13.3	13	14	14.8	13.9	14.6
5	12	14	12.8	13.7	15.7	14.6	14.7
6	13	14.7	14.6	13.3	16.7	16	15.7
7	15	14	12.7	13.7	16.3	16.8	15
8	12	15	13.1	13.7	16.3	16.1	16.8
9	15	12.5	12.4	14.2	16.9	16.5	15.9
10	14	12.7	12.8	13.7	16.8	13.7^{***}	16.9
11	15	17	13.1	16.3	16.8	14.7^{*}	14.3^{**}
12	12	16.3	12.8	16.3	16.3	16.3	14.7

Values were expressed as Mean. * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Table 3.2: Increase In Body Weight During 12 Weeks.

Week	Normal control	Control	Control + 200 mg	Control + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	298	302	303	301	291	295	288
2	313	311	312	313	300	301	295
3	328	319	325	329	325	320	315
4	339	312	337	335	343	331	330
5	344	321	331	339	345	332	312
6	349	331	337	336	351	343	334
7	350	334	350	348	355	350	341
8	355	359	362	355	376	369	366
9	359	354	368	351	374	372	366
10	361	358	369	365	389	379	371
11	366	377	371	368	399	381	385
12	378	381	385	381	415	405	406

Values were expressed as Mean. * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Table 3.1: Uterine weight

Animal group	Uterine weight (mg)
Normal Control	589 ± 18.05
Control	587 ± 23.54
Control+ 2%	523 ± 28.61
Control+5%	594 ± 24.12
Ovx	112*** ± 25.31
Ovx+2%	121 ± 40.97
Ovx+5%	115 ± 20.08

Values were expressed as Mean ± S.E.M.

* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Table 3.2 : Serum Calcium Profile

Week	Normal Control	Control	Control + 200 Mg	Control + 400 Mg	Ovx	Ovx + 200 Mg	Ovx + 400 Mg
1	9.710 ± 0.031	9.710 ± 0.223	9.710 ± 0.981	9.239 ± 0.304	9.810 ± 0.896	9.810 ± 0.459	9.810 ± 0.859
2	9.610 ± 0.400	7.968 ± 0.230	8.759 ± 0.860	9.019 ± 0.179	9.610 ± 0.444	6.707*** ± 0.569	7.407* ± 1.125
3	9.009 ± 0.260	7.808 ± 0.451	7.508 ± 0.667	8.679 ± 0.223	9.680 ± 0.587	8.609 ± 0.459	7.908 ± 0.239
4	8.008 ± 0.330	7.908 ± 0.280	7.508 ± 0.524	8.018 ± 0.124	9.209 ± 1.121	9.468 ± 0.154	8.909 ± 0.559
5	8.408 ± 0.055	9.875 ± 0.641	9.309 ± 0.248	8.238 ± 0.123	8.188 ± 0.997	9.220 ± 0.640	9.009 ± 0.690
6	9.009 ± 0.250	9.309 ± 0.731	9.229 ± 1.000	8.238 ± 0.340	8.018 ± 0.569	8.008 ± 0.258	8.108 ± 0.559
7	8.609 ± 0.230	9.309 ± 0.522	9.810 ± 0.899	8.128 ± 0.123	7.127 ± 0.700	7.608 ± 0.881	8.263 ± 0.700
8	8.809 ± 0.230	8.909 ± 0.459	8.809 ± 0.700	8.569 ± 0.213	6.126 ± 0.787	6.607 ± 0.230	8.464* ± 0.881
9	9.209 ± 0.320	9.239 ± 0.320	8.899 ± 0.230	8.599 ± 0.354	6.246 ± 0.230	7.127 ± 0.120	8.148 ± 0.230
10	9.409 ± 0.451	9.470 ± 0.230	8.679 ± 0.120	8.128 ± 0.267	6.106 ± 0.160	7.818 ± 0.230	8.298* ± 0.120
11	8.909 ± 0.340	9.369 ± 0.120	9.239 ± 0.340	8.899 ± 0.469	5.235 ± 0.190	7.137 ± 0.461	8.599*** ± 0.451
12	9.019 ± 0.110	9.129 ± 0.230	9.419 ± 0.230	9.019 ± 0.263	5.145 ± 0.280	7.197 ± 0.180	8.609*** ± 0.611

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

Table 3.3 Urinary calcium profile

Week	Normal	Control	C + 200 mg	C + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	3.78 ± 0.01	3.23 ± 0.07	2.97 ± 0.31	2.91 ± 0.30	6.01 ± 0.10	6.01 ± 0.15	6.01 ± 0.28
2	3.47 ± 0.13	3.78 ± 0.08	3.47 ± 0.29	3.40 ± 0.18	6.18 ± 0.06	6.12 ± 0.19	6.36* ± 0.38
3	4.00 ± 0.09	4.12 ± 0.15	3.78 ± 0.22	3.71 ± 0.22	6.66 ± 0.07	6.23 ± 0.15	5.89** ± 0.08
4	3.99 ± 0.11	4.01 ± 0.09	3.68 ± 0.17	3.61 ± 0.12	6.13 ± 0.04	6.01 ± 0.05	5.14*** ± 0.19
5	4.12 ± 0.02	4.14 ± 0.21	3.80 ± 0.08	3.73 ± 0.12	6.90 ± 0.04	5.89*** ± 0.21	5.23*** ± 0.23
6	3.66 ± 0.08	3.89 ± 0.24	3.57 ± 0.33	3.50 ± 0.34	7.10 ± 0.11	5.24*** ± 0.09	4.12*** ± 0.19
7	4.10 ± 0.08	4.19 ± 0.17	3.85 ± 0.30	3.77 ± 0.12	6.53 ± 0.04	5.14*** ± 0.29	4.39*** ± 0.23
8	3.56 ± 0.08	3.67 ± 0.15	3.37 ± 0.23	3.30 ± 0.21	6.44 ± 0.07	4.12*** ± 0.08	3.89*** ± 0.29
9	4.20 ± 0.11	4.19 ± 0.11	3.85 ± 0.08	3.77 ± 0.35	6.89 ± 0.12	4.13*** ± 0.04	3.86*** ± 0.08
10	3.18 ± 0.15	3.98 ± 0.08	3.65 ± 0.04	3.58 ± 0.27	7.24 ± 0.09	4.00*** ± 0.08	4.13*** ± 0.04
11	3.66 ± 0.11	3.49 ± 0.04	3.20 ± 0.11	3.14 ± 0.47	6.95 ± 0.16	5.12*** ± 0.15	3.66*** ± 0.15
12	3.54 ± 0.04	3.18 ± 0.08	2.92 ± 0.08	2.86 ± 0.26	6.66 ± 0.09	4.19*** ± 0.06	3.15*** ± 0.20

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

Table 3.4 Calcium Excretory rate

Week	Normal	Control	C + 200 mg	C + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	0.3892	0.3329	0.3057	0.3149	0.6122**	0.6122	0.6122
2	0.3611	0.4744	0.3963	0.3773	0.6431**	0.6125	0.8586
3	0.4440	0.5277	0.5039	0.4274	0.6880**	0.7237	0.7448
4	0.4984	0.5071	0.4905	0.4502	0.6651**	0.6348	0.5770**
5	0.4900	0.4192	0.4084	0.4524	0.8427**	0.6388	0.5805**
6	0.4063	0.4179	0.3870	0.4251	0.8855**	0.6543	0.5081**
7	0.4763	0.4501	0.3922	0.4641	0.9162**	0.6756**	0.5313**
8	0.4041	0.4119	0.3826	0.3856	1.0512**	0.6236*	0.4596**
9	0.4561	0.4535	0.4324	0.438	1.1031***	0.5795**	0.4737**
10	0.3380	0.4203	0.4211	0.4408	1.1857**	0.5117**	0.4977**
11	0.4108	0.3725	0.3469	0.3531	1.3276***	0.7174**	0.4257***
12	0.3925	0.3483	0.3100	0.3174	1.2944***	0.5816**	0.3658***

Values were expressed as Mean. * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Table 3.5: Hydroxyproline Excretory Rate

Week	Normal	Control	C + 200 mg	C + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	0.486 ± 0.010	0.416 ± 0.013	0.382 ± 0.016	0.394 ± 0.020	0.514 ± 0.016	0.514 ± 0.014	0.514 ± 0.012
2	0.451 ± 0.020	0.593 ± 0.025	0.495 ± 0.031	0.472 ± 0.039	0.540 ± 0.033	0.767 ± 0.028	0.722 ± 0.023
3	0.555 ± 0.012	0.660 ± 0.015	0.630 ± 0.019	0.534 ± 0.023	0.578 ± 0.020	0.608 ± 0.017	0.626 ± 0.014
4	0.623 ± 0.012	0.634 ± 0.015	0.613 ± 0.019	0.563 ± 0.023	0.559 ± 0.020	0.533 ± 0.017	0.485 ± 0.014
5	0.612 ± 0.098	0.524 ± 0.123	0.510 ± 0.153	0.566 ± 0.191	0.708 ± 0.161	0.537 ± 0.135	0.488 ± 0.114
6	0.508 ± 0.087	0.522 ± 0.109	0.484 ± 0.136	0.531 ± 0.170	0.637 ± 0.122	0.471 ± 0.088	0.366 ± 0.063
7	0.595 ± 0.087	0.563 ± 0.109	0.490 ± 0.136	0.580 ± 0.170	0.659 ± 0.122	0.486 ± 0.088	0.382 ± 0.063
8	0.505 ± 0.047	0.515 ± 0.059	0.478 ± 0.073	0.482 ± 0.092	0.756 ± 0.066	0.449 ± 0.048	0.331^{**} ± 0.034
9	0.570 ± 0.096	0.567 ± 0.120	0.540 ± 0.150	0.548 ± 0.187	0.794 ± 0.135	0.417^{*} ± 0.097	0.341^{**} ± 0.070
10	0.422 ± 0.049	0.525 ± 0.061	0.526 ± 0.076	0.551 ± 0.095	0.853 ± 0.068	0.368^{**} ± 0.049	0.358^{**} ± 0.035
11	0.514 ± 0.047	0.466 ± 0.059	0.434 ± 0.074	0.441 ± 0.092	0.928 ± 0.065	0.502^{**} ± 0.045	0.298^{***} ± 0.032
12	0.491 ± 0.098	0.435 ± 0.123	0.388 ± 0.153	0.397 ± 0.191	0.905 ± 0.134	0.407^{***} ± 0.094	0.256^{***} ± 0.065

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

Table 3.6: Serum AlP Profile.

Week	Normal	Control	C + 200 mg	C + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	57.488 ± 1.239	57.488 ± 1.652	49.559 ± 2.643	51.660 ± 2.974	161.592 ± 2.643	151.65* ± 1.652	150.659** ± 3.965
2	58.713 ± 1.248	57.480 ± 3.304	58.480 ± 2.974	55.873 ± 1.652	160.571 ± 3.634	144.573*** ± 3.634	152.641 ± 3.634
3	56.049 ± 1.972	53.524 ± 2.643	54.515 ± 3.634	57.052 ± 1.982	157.597 ± 1.322	148.042* ± 2.974	120.924*** ± 2.313
4	53.179 ± 0.258	55.506 ± 1.652	55.50 ± 3.965	54.436 ± 1.322	158.589 ± 2.643	134.969*** ± 1.652	117.950*** ± 1.652
5	60.485 ± 0.981	47.577 ± 1.982	53.524 ± 3.304	45.158 ± 3.634	151.650 ± 2.974	133.343*** ± 1.322	131.827*** ± 2.974
6	57.612 ± 0.261	45.594 ± 2.974	50.669 ± 1.322	42.601 ± 1.982	141.739 ± 2.313	126.782*** ± 3.634	98.751*** ± 2.643
7	56.748 ± 0.153	45.594 ± 3.634	47.577 ± 1.652	40.490 ± 3.634	136.783 ± 1.652	116.444*** ± 2.974	90.842*** ± 1.982
8	50.783 ± 0.981	53.524 ± 2.643	53.662 ± 2.643	40.589 ± 2.974	132.808 ± 2.643	117.237*** ± 1.982	92.794*** ± 3.634
9	51.961 ± 1.242	54.515 ± 1.239	55.506 ± 1.635	45.822 ± 1.219	143.721 ± 1.675	114.213*** ± 1.616	81.396*** ± 1.635
10	55.202 ± 1.443	58.480 ± 1.279	56.497 ± 1.873	49.123 ± 1.635	137.774 ± 1.517	109.149*** ± 1.675	77.956*** ± 1.219
11	54.838 ± 1.485	60.462 ± 1.616	53.524 ± 1.635	42.740 ± 0.228	133.809 ± 1.219	103.311*** ± 1.665	65.646*** ± 0.882
12	58.602 ± 1.254	56.497 ± 1.626	58.480 ± 1.219	42.185 ± 1.517	131.827 ± 1.616	97.760*** ± 2.309	67.400*** ± 0.654

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

Table 3.7: Serum TRAcP profile

Week	Normal	Control	C + 200 mg	C + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	8.90 ± 0.53	8.30 ± 0.56	7.80 ± 0.88	7.60 ± 0.91	12.60 ± 0.90	12.50 ± 0.56	11.40 ± 0.97
2	8.60 ± 0.96	7.80 ± 0.12	7.20 ± 0.90	6.20 ± 0.54	12.40 ± 0.90	11.00 ± 0.90	9.90 ± 0.85**
3	8.10 ± 0.79	7.60 ± 0.89	6.30 ± 0.12	5.20 ± 0.66	14.60 ± 0.91	9.10 ± 0.46***	9.60 ± 0.75
4	8.30 ± 0.13	6.80 ± 0.53	5.20 ± 0.12	5.30 ± 0.43	16.50 ± 1.24	9.60 ± 0.79***	9.85 ± 0.87***
5	8.20 ± 0.12	7.80 ± 0.63	5.40 ± 0.20	5.40 ± 0.11	16.80 ± 1.10	9.40 ± 0.13***	8.63 ± 0.46*
6	8.60 ± 0.46	7.60 ± 0.91	5.70 ± 0.46	5.60 ± 0.66	17.10 ± 1.00	9.20 ± 0.97***	8.12 ± 0.79*
7	8.90 ± 0.57	7.60 ± 0.11	5.80 ± 0.54	5.70 ± 0.23	17.90 ± 0.98	8.90 ± 0.87***	7.90 ± 0.99***
8	8.10 ± 0.86	7.90 ± 0.83	5.90 ± 0.53	5.80 ± 0.91	18.20 ± 0.78	8.80 ± 0.43***	7.62 ± 0.75***
9	7.90 ± 0.75	8.10 ± 0.83	5.86 ± 0.65	5.42 ± 1.22	18.63 ± 0.96	9.63 ± 0.25***	7.12 ± 0.79***
10	8.30 ± 0.45	8.02 ± 0.63	5.98 ± 0.23	5.26 ± 0.69	18.39 ± 1.23	9.23 ± 0.26***	6.85 ± 0.23***
11	8.00 ± 0.15	8.60 ± 0.89	6.12 ± 0.45	6.12 ± 0.25	19.23 ± 1.23	10.13 ± 0.45***	7.25 ± 1.26***
12	8.50 ± 0.15	8.30 ± 0.12	6.23 ± 0.12	5.23 ± 0.15	19.99 ± 0.62	11.23 ± 1.23***	7.63 ± 0.62***

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

Table 3.8 Mean Food intake

Week	Normal	Control	C + 200 mg	C + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	34.032	26.003	23.413	25.226	26.78	26.521	25.485
2	34.032	30.665	34.55	33.773	33.255	33.255	30.665
3	31.442	34.032	34.55	35.845	36.104	34.032	37.399
4	36.622	34.809	34.032	36.622	38.694	36.363	38.176
5	31.442	36.622	33.514	35.845	41.025	38.176	38.435
6	34.032	38.435	38.176	34.809	43.615	41.802	41.025
7	39.212	36.622	33.255	35.845	42.579	43.874	39.212
8	31.442	39.212	34.291	35.845	42.579	42.061	43.874
9	39.212	32.737	32.478	37.14	44.133	43.097	41.543
10	36.622	33.255	33.514	35.845	43.874	35.845	44.133
11	39.212	44.392	34.291	42.579	43.874	38.435	37.399
12	31.442	42.579	33.514	42.579	42.579	42.579	38.435

Values were expressed as Mean.

Table 3.9: Calcium absorption rate (%)

Week	Normal	Control	C + 200 mg	C + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	71.37518	47.81756	42.66006	47.43915	44.82636	54.25512	55.30704
2	71.37518	55.75086	61.14327	60.74083	55.56909	63.51827	62.85668
3	67.36976	60.1287	61.14327	63.01018	59.07517	64.3512	69.54464
4	74.81405	61.0187	60.55183	63.79499	61.81449	66.63642	70.1645
5	67.36976	62.9485	59.94211	63.01018	63.98416	68.22087	70.36555
6	71.37518	64.69624	64.83393	61.90928	66.12289	70.97747	72.23644
7	77.79863	62.9485	59.63013	63.01018	65.29862	72.34809	70.95277
8	67.36976	65.3958	60.84979	63.01018	65.29862	71.15618	74.03929
9	77.79863	58.55149	58.66433	64.29995	66.52052	71.84955	72.58263
10	74.81405	59.19711	59.94211	63.01018	66.32288	66.15428	74.19165
11	77.79863	69.43368	60.84979	68.86024	66.32288	68.43502	69.54464
12	67.36976	68.13218	59.94211	68.86024	65.29862	71.50708	70.36555

Values were expressed as Mean.

Table 3.10 calcium retention rate

Week	Normal	Control	C + 200 mg	C + 400 mg	OVX	OVX + 200 mg	OVX + 400 mg
1	91.3	85.837	82.369	85.285	78.875	79.86	81.69
2	91.3	89.699	91.664	91.416	86.277	85.36	74.220**
3	90.024	91.394	91.664	92.203	76.123	86.120*	85.23
4	92.287	91.709	91.454	92.462	79.563	82.16	84.63
5	90.024	92.361	91.234	92.203	78.254	86.23	78.23
6	91.3	92.918	92.885	91.828	70.235	89.120**	79.630*
7	93.073	92.361	91.12	92.203	74.236	82.13	81.63
8	90.024	93.133	91.56	92.203	74.236	84.123*	82.63
9	93.073	90.813	90.757	92.626	78.653	86.12	89.360**
10	92.287	91.055	91.234	92.203	71.236	84.230***	94.260**
11	93.073	94.287	91.56	93.994	74.236	89.630***	89.360***
12	90.024	93.93	91.234	93.994	71.026	85.120***	91.230***

Values were expressed as Mean. * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Table 3.11: Bone calcium content

Experimental group	Bone calcium content (mg/gm of bone)
Normal Control	19.6 ± 1.09
Control	21.3 ± 1.423
Control+ 2%	21.9 ± 1.23
Control+5%	20.2 ± 1.09
Ovx	14.5 ± 2.3***
Ovx+2%	15.2 ± 2.19
Ovx+5%	16.9 ± 1.11*

Values were expressed as Mean ± S.E.M.

* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

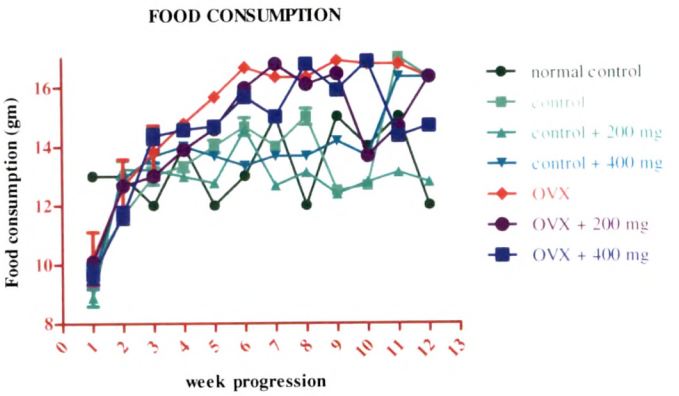


Figure 3.1 Food consumption for 12 weeks.

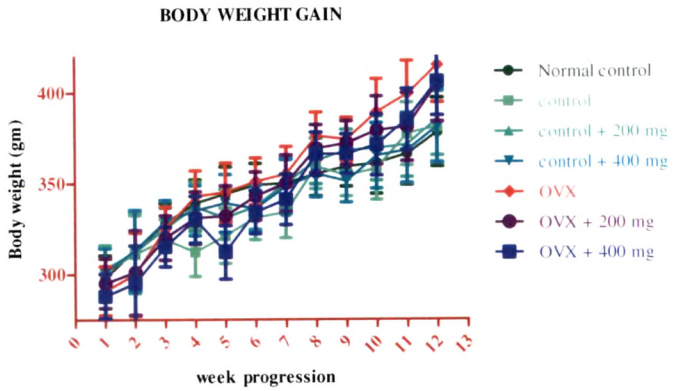


Figure 3.2 Body weight gain in 3 months of study period.

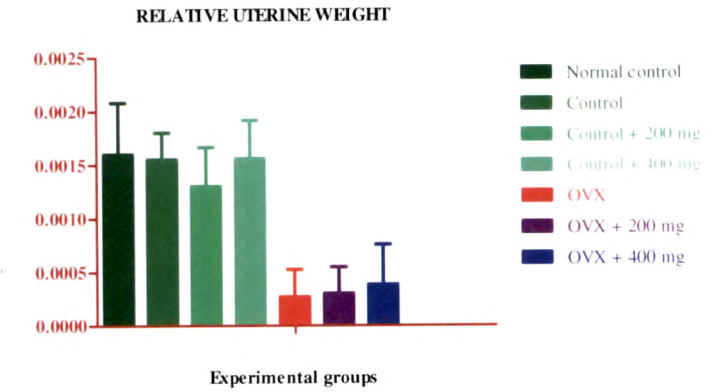


Figure 3.3 Relative uterine weight of animals.

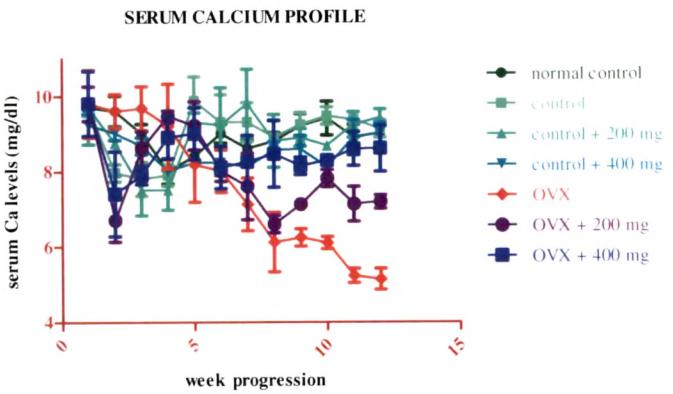


Figure 3.4 Serum calcium profile

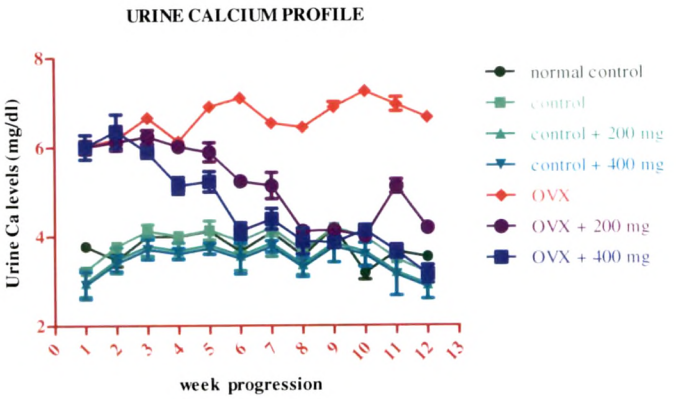


Figure 3.5 Urine calcium Profile

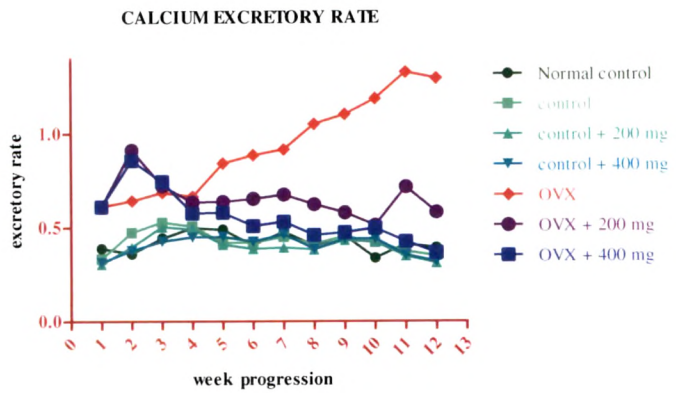


Figure 3.6 Calcium Excretory rate.

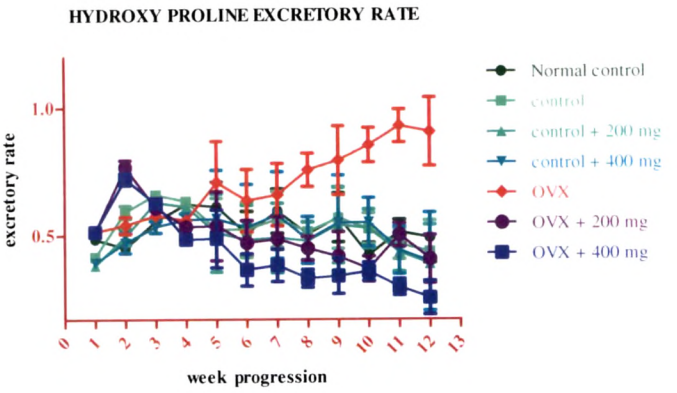


Figure 3.7 Hydroxyproline excretory rate.

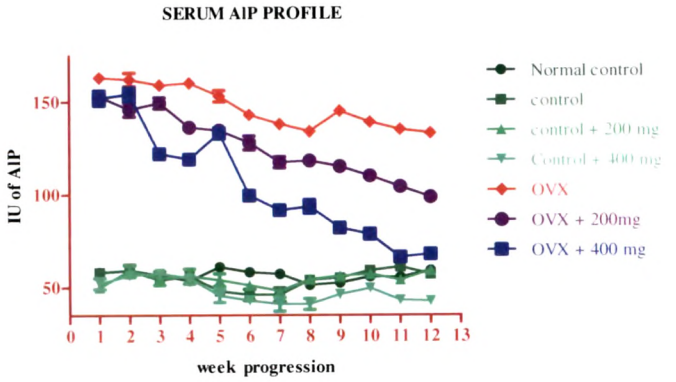


Figure 3.8 Serum AlP profile

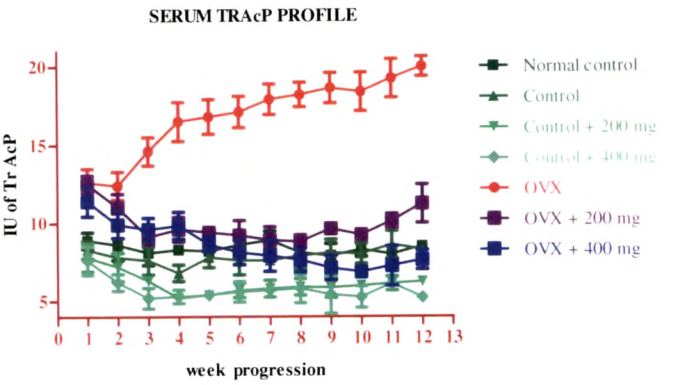


Figure 3.9 Serum TRAcP profile

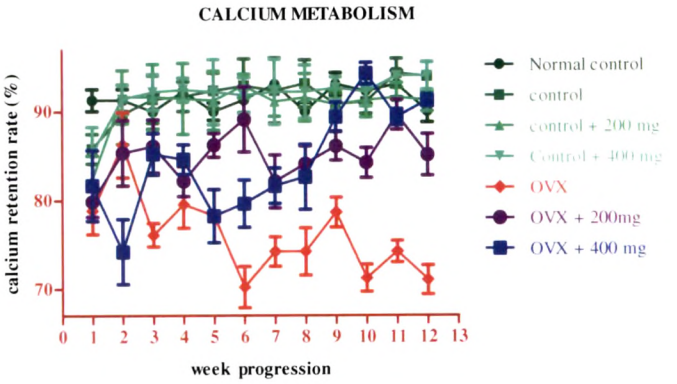


Figure 3.10 Calcium Metabolism

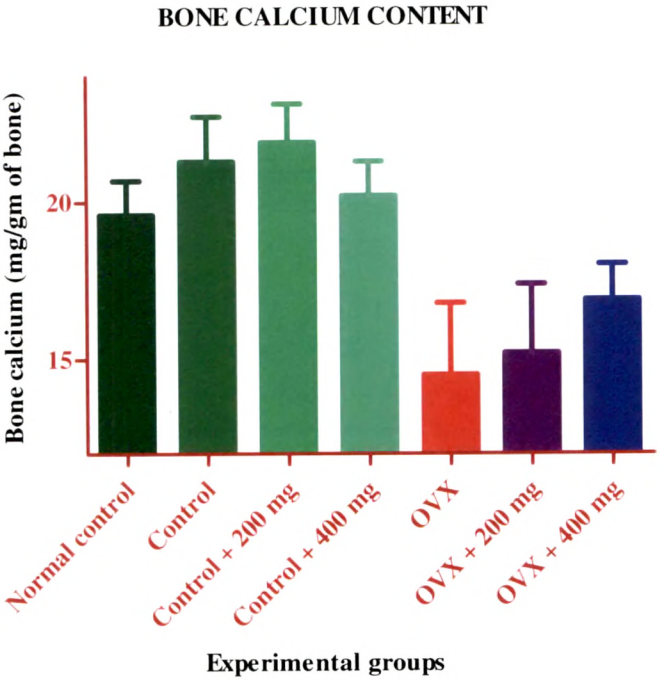
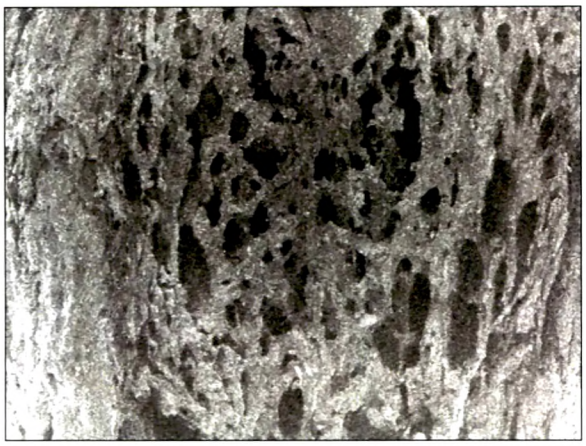


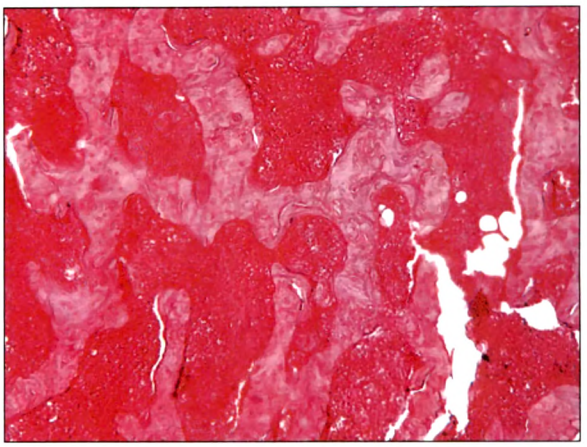
Figure 3.11 Bone calcium content.

SCANNING ELECTRON MICROSCOPY

LIGHT MICROSCOPY

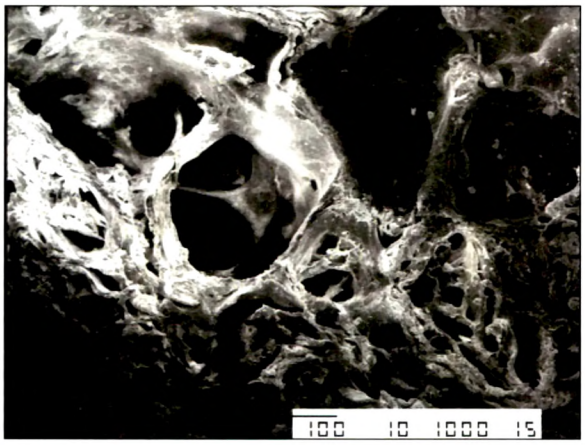


Normal cancellous bone

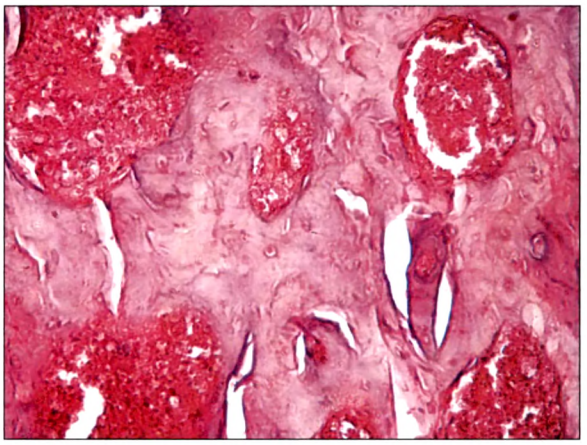


Normal cancellous bone (100X)

Figure 3.12a Control bone histoarchitecture at low magnification showing intact cancellous bone



Cancellous bone showing trabecules

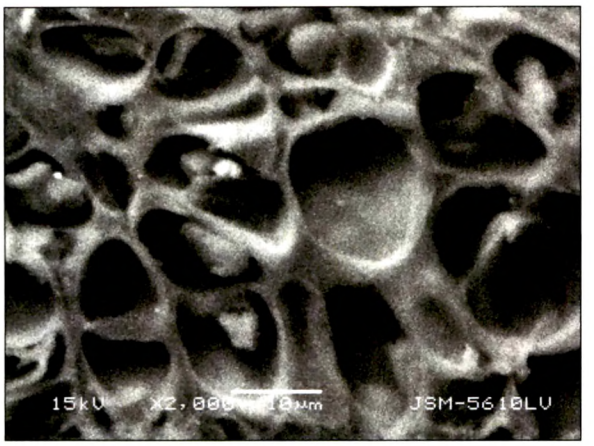


Cancellous bone showing trabecules (200X)

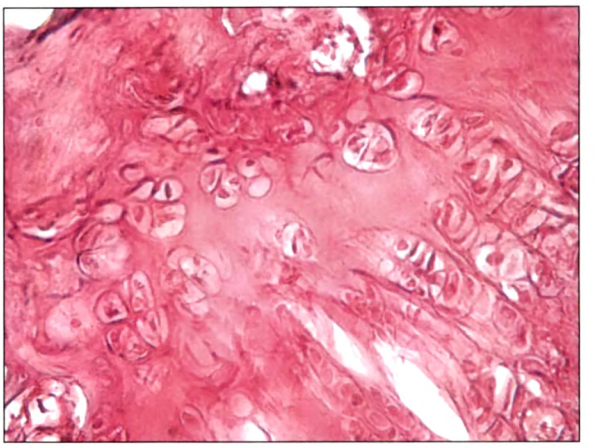
Figure 3.12b Control bone histoarchitecture showing intact trabecules and bone marrow.

SCANNING ELECTRON MICROSCOPY

LIGHT MICROSCOPY

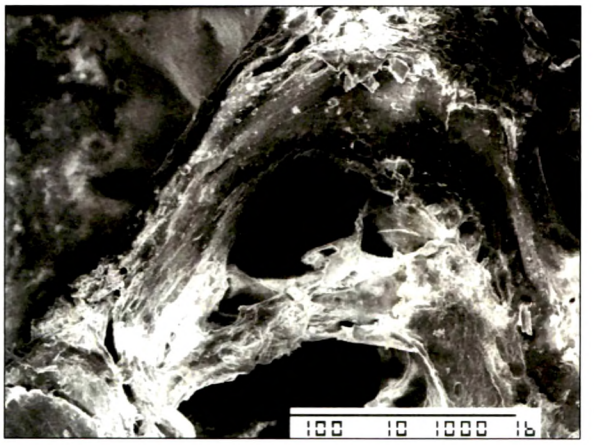


Epiphseal growth plate

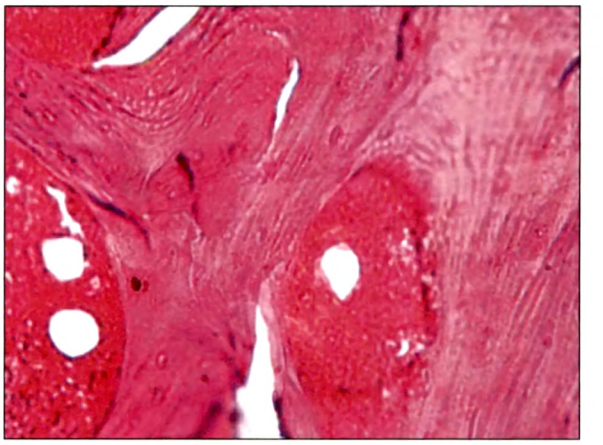


Epiphseal growth plate

Figure 3.12c Control bone histoarchitecture at high magnification showing well marked epiphyseal growth plate



Cancellous bone at high magnification

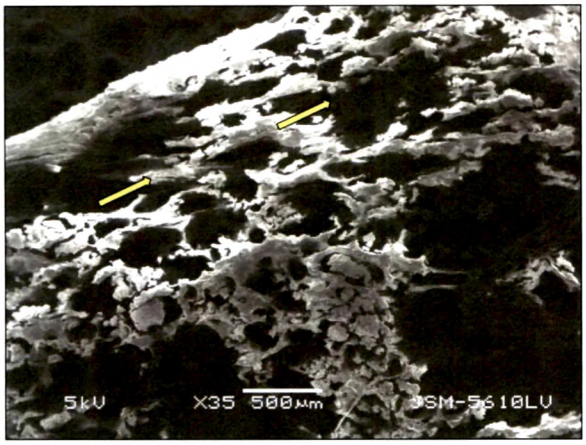


Cancellous bone at high magnification (400X)

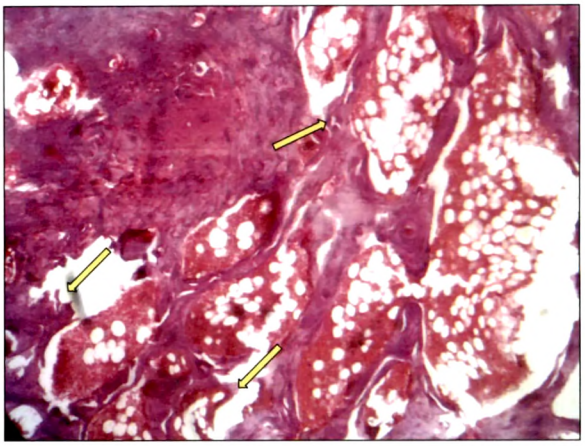
Figure 3.12d showing Control bone histoarchitecture at high magnification indicating thick trabecules

SCANNING ELECTRON MICROSCOPY

LIGHT MICROSCOPY

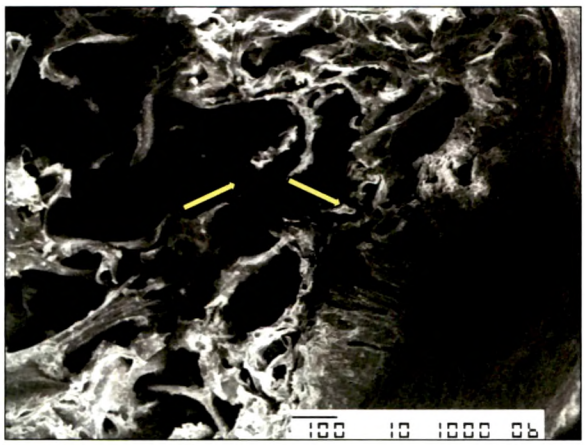


Osteoporotic cancellous bone

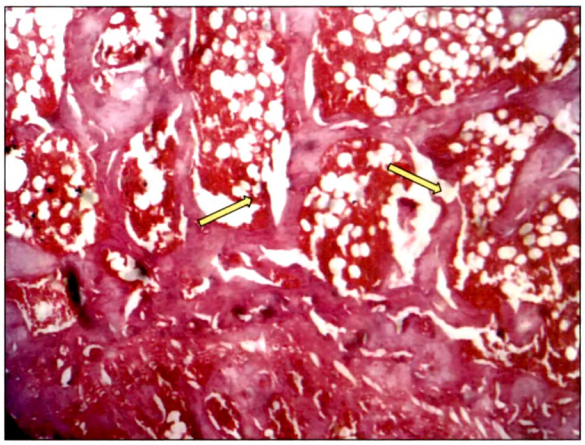


Osteoporotic cancellous bone (100X)

Figure 3.13a OVX bone histoarchitecture at low magnification showing damaged (⟹)cancellous bone



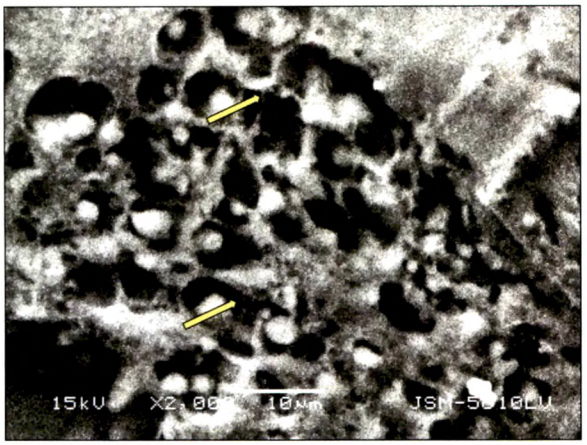
Cancellous bone showing damaged trabecules

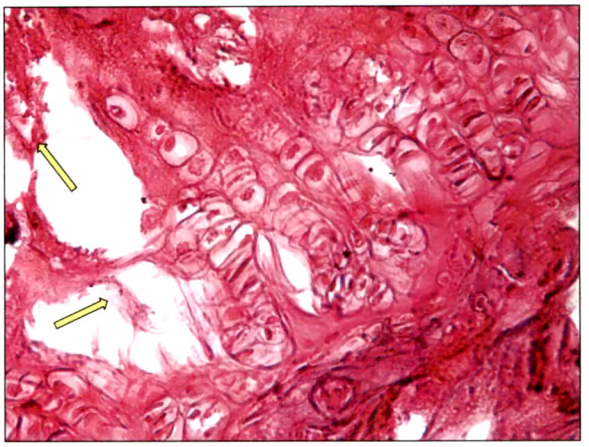


Cancellous bone showing damaged trabecules (200X)

Figure 3.13b OVX bone histoarchitecture showing damged trabecules(⟹)and scattered bone marrow.

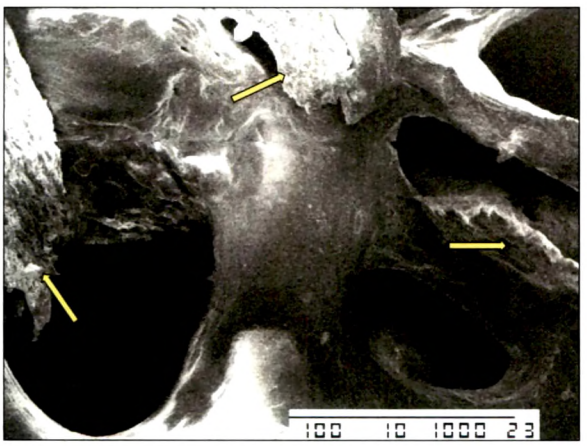
SCANNING ELECTRON MICROSCOPY LIGHT MICROSCOPY

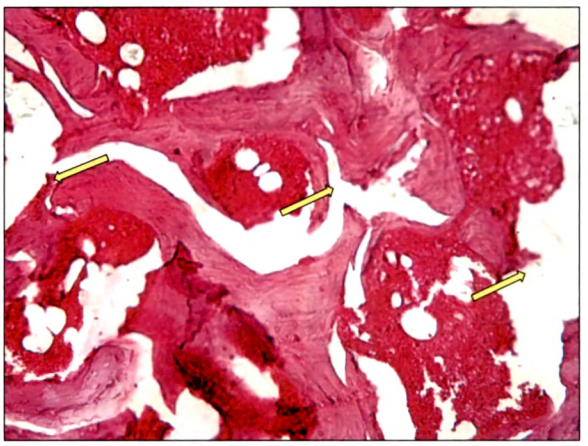




Excessive resorption near epiphyseal growth plate Excessive resorption near epiphseal growth plate (400X)

Figure 3.13c OVX bone histoarchitecture at high magnification showing resorption(⟹)at epiphyseal growth plate



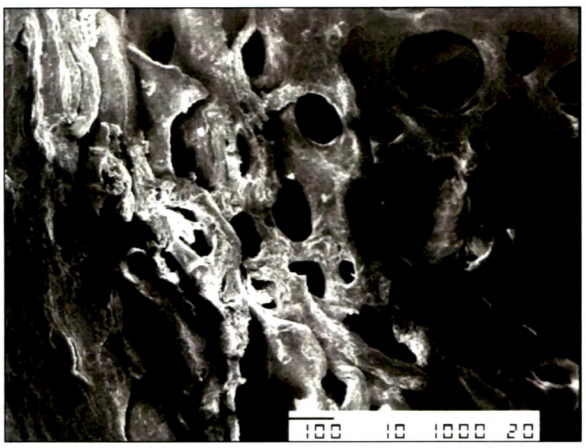


Osteoporotic cancellous bone at high magnification Osteoporotic cancellous bone(200X)

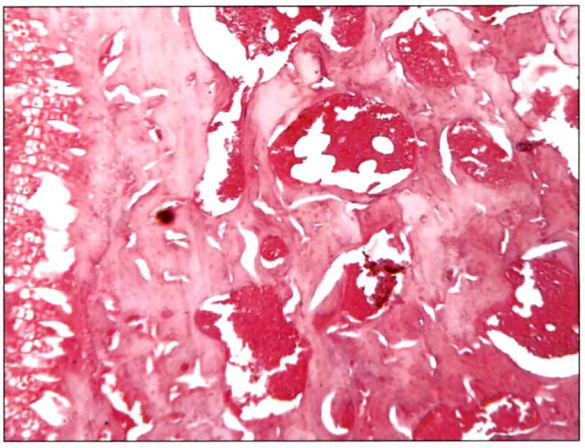
Figure 3.13d showing OVX bone histoarchitecture at high magnification indicating thin and damaged(⟹)trabecules

SCANNING ELECTRON MICROSCOPY

LIGHT MICROSCOPY

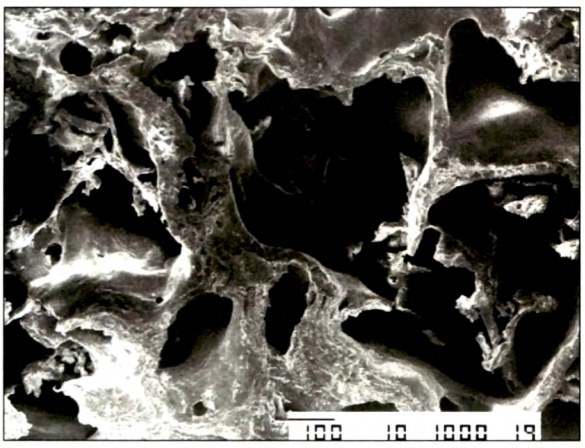


Osteoporotic cancellous bone

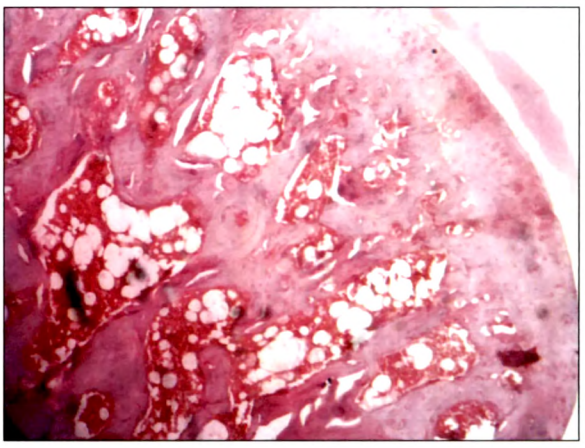


Osteoporotic cancellous bone (100X)

Figure 3.14a showing improvement in bone histoarchitecture by 200 mg/kg bw LG treatment



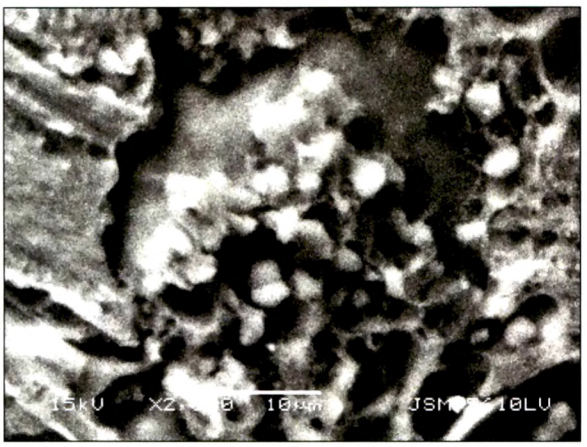
Cancellous bone showing damaged trabecules



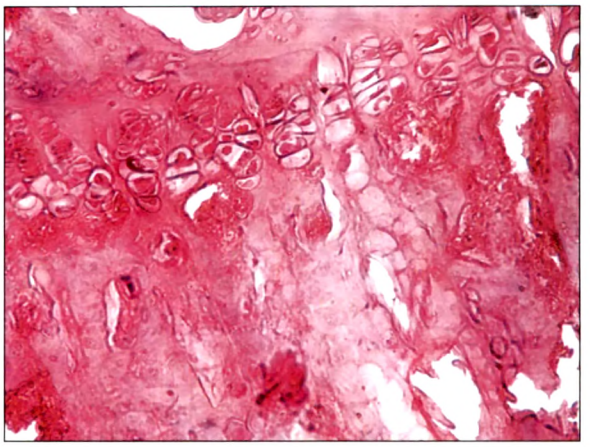
Cancellous bone showing damaged trabecules (100X)

Figure 3.14b Showing improvement in bone histoarchitecture by 200 mg/kg bw LG treatment

SCANNING ELECTRON MICROSCOPY LIGHT MICROSCOPY

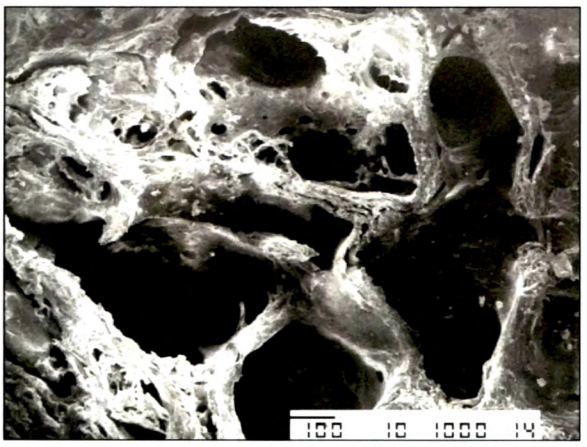


Reduced resorption near growth plate

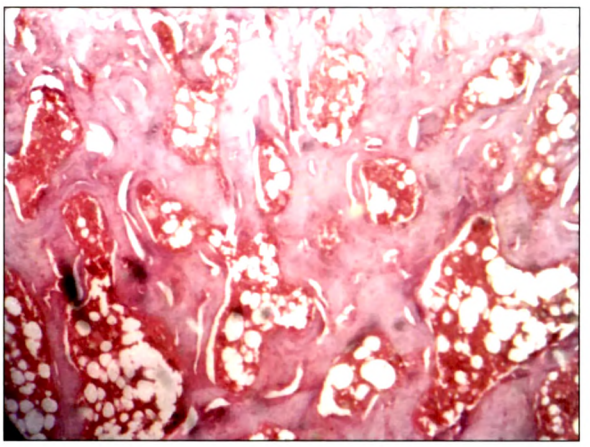


Reduced resorption near growth plate (400X)

Figure 3.14c Showing reduced resorption at epiphyseal growth plate with 200 mg/kg bw treatment of LG.



Improved cancellous bone at magnification

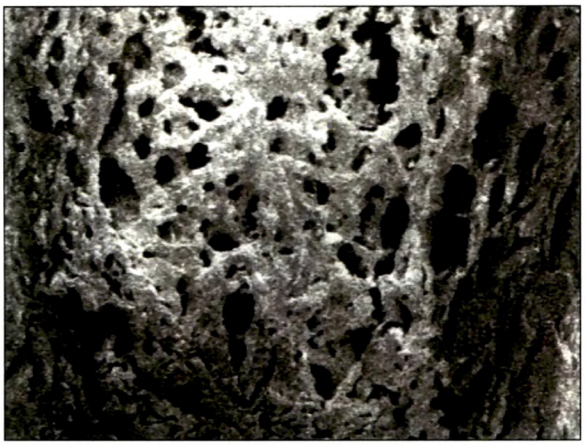


Improved cancellous bone at magnification (200X)

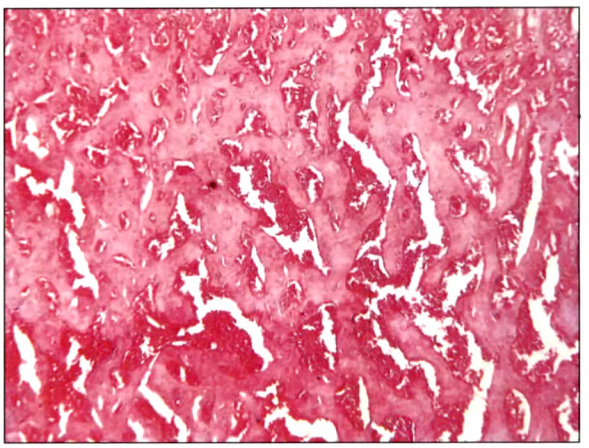
Figure 3.14d showing partial restoration of damaged trabecules with 200mg/kg bw of LG treatment.

SCANNING ELECTRON MICROSCOPY

LIGHT MICROSCOPY

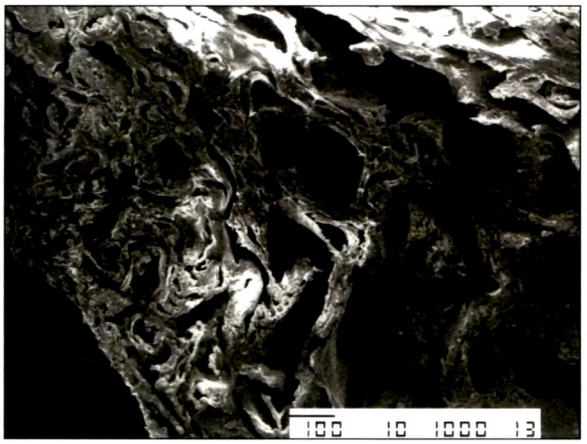


Cancellous bone

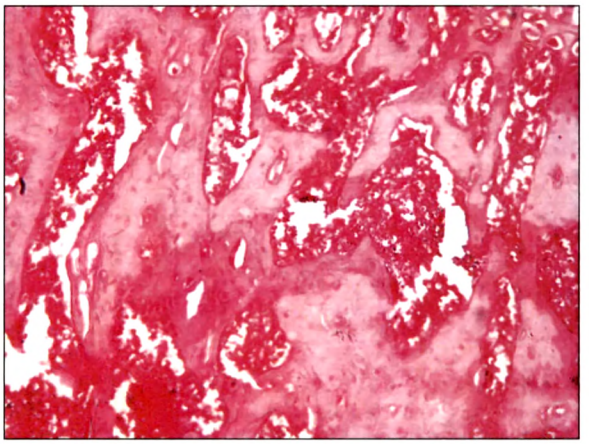


Cancellous bone (100X)

Figure 3.15a showing improvement in bone histoarchitecture by 400 mg/kg bw LG treatment



Cancellous bone showing restoration in trabecules

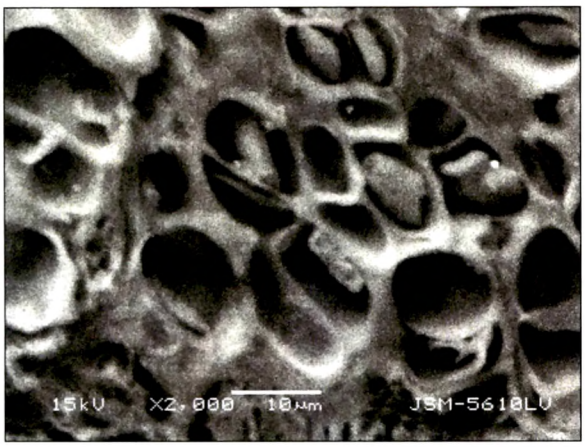


Bone showing restoration of trabecules (200X)

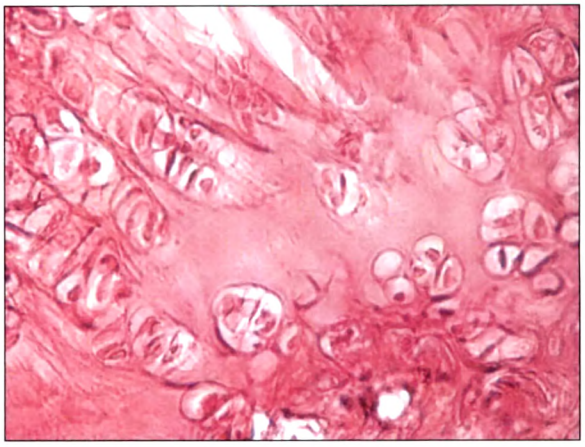
Figure 3.15b Showing improvement in bone histoarchitecture by 400 mg/kg bw LG treatment

SCANNING ELECTRON MICROSCOPY

LIGHT MICROSCOPY

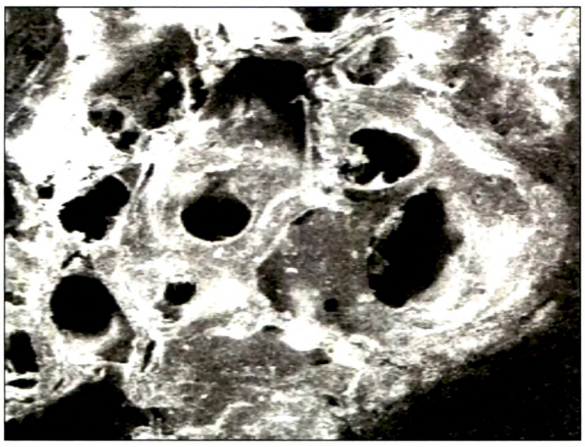


Reduced resorption near growth plate

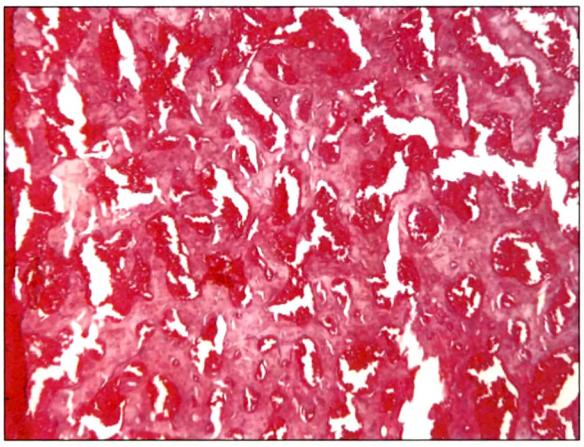


Reduced resorption near growth plate (400X)

Figure 3.15c Showing negligible resorption at epiphyseal growth plate with 400 mg/kg bw treatment of LG.



Cancellous bone at magnification



Cancellous bone at magnification (200X)

Figure 3.15d showing complete restoration of damaged trabecules with 400mg/kg bw of LG treatment.