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THE EFFECT AND ESTIMATION OF "RALOXIFENE (IN ADDITION TO CALCIUM AND VITAMIN D3 SUPPLEMENTATION) ON AEDS"-INDUCED CHANGES IN BONE MINERAL DENSITY AND BONE TURNOVER" MARKERS

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Abstract

Antiepileptic medications are frequently required for the rest of a person's life if they suffer from epilepsy, a common neurological illness (AEDs). Recent decades have shown that long-term use of AEDs can lead to bone loss and a greater risk of fracture. Long-term use of AEDs has been shown to decrease BMD, increase the risk of fracture, and even cause overt osteomalacia, according to new clinical data AED-related bone fragility may be caused by a variety of mechanisms, but the most common AEDs that have been linked to bone metabolism disorders are CYP 450 monoxygenase system inducers (phenytoin, carbamazepine, and phenobarbitone) Thus, the present study was designed. the effect of "raloxifene (in addition to calcium and vitamin D3 supplementation) on AEDs"-induced changes in bone mineral density and bone turnover" markers. And estimation of hydroxyproline, calcium and Creatinine.

Keywords: Epilepsy, AED: Anti-epileptic drugs therapy CYP

Introduction:

Epilepsy is a common illness that normally needs lifetime treatment with antiepileptic medications (AEDs). In the last four decades, the use of prolonged AED medication has been consistently linked with a lower bone density and increased risk of fracture (Kruse, 1968; Lee et al., 2010; Petty et al., 2007). Recent clinical data has also verified prior studies showing that the long-term treatment of AODs affects bone mineral density (BMD) (Shiek et al., 2012; Espinosa et al., 2011). AED-induced effects on the bone Prevalence rates of 50 percent or more were found (Valsamis et al., 2006). The bone microarchitecture and BMD are therefore increased by the occurrence of fractures, not just AED, but also by the illness itself (Valsamis et al., 2006; Pack et al., 2008). Multiple variables can help and, thus, the gross alterations and an increased fracture risk are not only caused by AED but also due to the seizures, traumas and sedentary lifestyles connected with the seizure activities (Khanna" et al., 2009).

"While the mechanisms responsible for the bone fragility associated with AED are probably multiple and yet poorly understandable, the AEDs that are most often reported to cause bone metabolist disorders are strong inducers of the Cytochrome P450 (CYP 450) monoxygenase system (phenytoin; carbamazepine; phenobarbitone) (Valsamis et al., 2006; Khanna et al., 2009). Although this is the major and most prevalent AED-induced bone loss mechanism, the same cannot always be said about different causes; firstly, vitamin D deficiencies are found in every patient with good deficiencies (Pack et al., 2011) Second, enzyme inhibitors AEDs were also connected with osseous adverse effects (Boluk et al., 2004). Other mechanisms, such as hypovitaminosis K, calcitonin insufficiency, lower intestinal calcium absorption and hyperhomocysteinemia may contribute to deleterious effects on bone levels, documented after treatment with AED (Fitzpatrick, 2004; Khanna et" al., 2009, 2011).

"Despite the introduction of second and third generation AEDs in the past decades, phenytoin (PHT) and sodium valproate (SVP) are still considered to be the first line drugs and are widely prescribed in the management of partial seizures and generalized tonic-clonic seizures (Nolan et al., 2013). Ample amount of literature is available on the adverse consequences of these AEDs on bone (Boluk et al., 2004; Khanna et al., 2009; Lee et al., 2010; Pack et al., 2011). The evidence on newer AEDs affecting bone metabolism is limited and needs to be investigated though there have been few reports of gabapentin, lamotrigine topiramate, and vigabatrin affecting bone turnover in epileptic patients (Khanna et al., 2009; Lee et al., 2012; Sheth and" Hermann, 2007).

"Levetiracetam (LTM) is one of the most often prescribed medications for handling partial and widespread seizures (Lyseng-Williamson, 2011). Although levetiracetam is increasingly used in both monotherapy and polytherapy, few studies have assessed the effect it has on BMD in individuals with an epilepsy. These investigations have produced inconsistent results, either finding no effects on bone metabolism or BMD in epileptic patients (Koo et al., 2013) or lower bone biomechanical force in animal trials without any major changes in BMD (Nissen-Meyer et" al., 2007).

"We require relevant animal models in order to evaluate the pathophysiology of AED-induced kidney alterations and to investigate the effectiveness of ant osteoporotic regimens in epilepsy. Although animal model osteoporosis in mice with glucocorticoids (Yang et al., 2009) or ovariectomy are available in most of the previous research, rats have been utilised to explore the impact of AED on bones (Nissen-Meyer et al., 2007; Onodera et al., 2002). (Bouxsein et al., 2005). Reciently, after 35mg/kg in our laboratory, we have developed a 35mg/kg po bone demineralization model in male mice (Khanna et al., 2011).

In this study we have examined if the effects of this medicine on BMD and ostrich markers in the mice may be induced after prolonged administration by sodium valproate (SVP) or LTM. In this investigation, there are only very few animal studies. Further, as we picked female mice to establish potential sexual impacts of antiepileptic medications on bone in the course of the investigation as most of the prior animal research were done with AEDs on the male" animal.

Literature Review:

Epilepsy "is one of the most frequent brain neurology diseases. Since 500 BC, the term epilepsy has been used and literally means attack. It is chronic and is marked as repeated and unprovoked seizures of the epileptics [ILAE, Fisher et al., 2005] commission report. An epileptic seizure is brief indications and/or symptoms caused to aberrant or aberrant neuronal activity in the brain (Fisher et al., 2005). In general, epilepsy has neuroscience, cognitive, psychological and social effects (Fisher et al., 2005). In the first year of life and in older persons over 65 years, it affects individuals of all categories (Hauser et al., 1993; Olafsson et" al., 2005).

The "prevalence of active epilepathy in affluent nations is 0.7 percent (Hauser et al, 1996; Keränen and Riekkinen, 1988), whereas the incidence is roughly 50 per 100,000 people (Hauser et al., 1996; Olafsson et al., 2005). It affects 50 million individuals throughout the world, and 80% of them reside in poorer countries (Meyer et al., 2010). In India the prevalence rate is, however, about 5/1000 (at this present rate the estimated epileptic population in this nation is around 5 million) and the incidence rate of two community studies varies between 38 and 49,3 for every 100,000 people each year (Ray et" al., 2002; Sridharan, 2002)"

Treatment of epilepsy

Antiepileptic "the key elements behind the treatment of epilepsy are antiepileptic medicines (AEDs), and though their number has increased exponentially, current guidelines on medical treatment are much the same as they were developed a century ago (Shorvon, 2009). As the treatment of AED is often continued for several years and frequently for life, especially for adults, a treatment decision has far-reaching effects and must be founded on comprehensive risk benefits analyses (Perucca et" al., 2000).

The "ultimate purpose of epilepsy is to avoid seizures without detrimental consequences. Therefore, first AED selection should largely be guided by evidence of effectiveness and tolerability for seizure type or epileptic syndrome of the" patient.

Estrogen, "TGF β3 and AED-induced effects on bone"

Estrogens "are widely established to have a crucial function in bone health. Not only do estrogens reduce cytocine levels (Interleukins, L-6, L-7; Tumor Necrosis Factor, TNF-α), which recruit osteoclasts (Vaananen and Harkonen, 1996,) but also contrast parathyroid hormone mobilising measures (Transforming growth factor, TGFβ3, which is a Bone Matrix Protein, with anti-osteoclate differentiation, and increase osteoblast differentiations by regulating the growth factor (Robinson et al., 1996). Last play a major role in modulating bone density by maintaining the equilibrium between osteoblast formation of the bone matrix and its osteoclastic resorption (Grainger et al., 1999). In addition, oestrogen shortage conditions

have previously been demonstrated to diminish TGF-β accumulation in rat bones (Finkelman et" al., 1992).

OBJECTIVES:

- To "determine the effect of "raloxifene (in addition to calcium and vitamin D3 supplementation) on AEDs"-induced changes in bone mineral density and bone turnover" markers.

- Bone Mineral Density and Bone Turnover" Markers

METHODOLOGY:

Bone Mineral Density and Bone Mineral Content analysis by DEXA :

"The left femora and lumbar vertebrae (L2-L4) were cleaned of soft tissue and then frozen at - 80˚C. Before the DEXA scan for BMD analysis the bones were defrosted for 30 minutes".

Sample preparation:

Bone tissue sample preparation

"Bones were split out of the lumbar vertebrae" (L2-L4) and muscles and tissues around them were removed. With 10 tanks of 10 mM triethanolamine buffer, the bones were weighed and homogenised (pH 7.5). The homogeneous product has been mixed and centrifuged for 1.5 hours at 4 BC. Aliquots of bone extracts were utilised to identify alkaline phosphatase (ALP) and tartrate "resistant phosphatase (TRAP) for two" times the removal procedure was repeated. The insoluble pellets were to be 24 hours hydrolyzed at a rate of 105 μC with 6N HCl".

Serum preparation

Each animal's blood obtained (2.5 times the volume required for usage) was incubated at room temp for 45 minutes in an upright position. The samples were centrifuged for 10 min, "in 3,000 rpm, once the coagulation has retreated". The resulting serum was kept at -20 € until further usage till future usage.

Urine Collection

"The urine collection was made in a clean bottle on ice for 24 hours and kept at -20 °C until further usage".
Histopathological studies (Belur et al., 1990)
1. **Decalcification:** "The Discomposed and fixed in 10% tamponed" formaline solution were the appropriate femoral and lumbar vertebrae. The medium acid (5 percent nitric acid with 0.5 percent urea) then was decalcified for 24 hours".

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2. **Dehydration:** The tissue's water content has been replaced by rising ethanol levels.

80% alcohol: 1 hr
95% alcohol: 1 hr
100% alcohol: 1 hr

Clearing: "Chloroform was utilised as a clearing agent for tissue clearance and transparent, signalling process completion".

Pharmacological Evaluation			
1.	Maximal electroshock induced seizure (MES)	-	Uma Devi et al., 2011
Bone Studies			
1.	Histopathology	Femur & Lumbar	Belur et al., 1990
2.	Bone Mineral Density (BMD)	Femur & Lumbar	-
Bone Turnover Markers			
1.	Alkaline phosphatase (ALP)	Lumbar (L2-L4)	Kind and King, 1954
2.	Tartrate Resistant Acid Phosphatase (TRAP)	Lumbar (L2-L4)	Tenniswood et al., 1976
3.	Hydroxyproline (HxP)	Lumbar (L2-L4)	Bergman and Loxley, 1963
4.	Calcium (Ca-U)	Urine	Pollard and Martin, 1956
Related Markers			
1.	Estradiol (E2)	Serum	-
2.	Transforming Growth Factor-β3 (TGF-β3)	Lumbar (L2-L4)	-

3. **Impregnation:** "Paraffin is used to completely remove the" cleaning agent, as it enters the tissues. Three-paraffin baths were performed for 3 hours, at 56 os-58 os os-cap. The tissue was then thrown into paraffin wax blocks".

4. **Sectioning:** "Sections of the 3-5µ thickness of the tissue" block were cut using rotary microtomes. The ribbons were designed to float in water, and later on glass toboggans".

5. **Hydration:** "The Sections of xylene were hydrated for 2 minutes and alcohol for 70% for 2 minutes. They were then washed with water that was distilled".

6. Staining: "Sections were stained with 1% heamatoxylin", washed with distilled water, and then added eosin 1% to 90% alcohol for 1 minute and dried slides.

7. Glycerin jelly "was coated in the stained parts and" cover slips were placed on the sections to ensure that there should be no air bubble entry. The diapositives were detected and photos were taken afterwards.

3. METHODS FOR ESTIMATIONS

Estimation of Hydroxyproline Method: Bergman and Loxley, 1963
Principle: "It includes oxidising free hydroxyproline (HxP) " to pyrolea with chloramine T and producing a "pink" pyroleum reaction with p-dimethylaminobenzaldehyde. Then the colour is spectrometrically measured at 562 nm".

Reagents:
1. 6N HCl: 555.56ml of 10.8N HCl was diluted to make the final volume 1000 ml with 68 double distilled waters.
2. HCl (1mM): 0.092ml of 10.8N HCl was diluted to make the final volume 1000 ml with double distilled water.

3. n-propanol

4. Chloramine-T reagent (7%): 7 g chloramine-T was dissolved in double distilled water and the volume was made upto 100 ml with double distilled water.

5. Citrate buffer (0.1M): 2.65 g citric acid was dissolved in 1000 ml double distilled water. 25.7 g sodium citrate was dissolved in 1000 ml double distilled water. Mixed both the solution in equal volume.

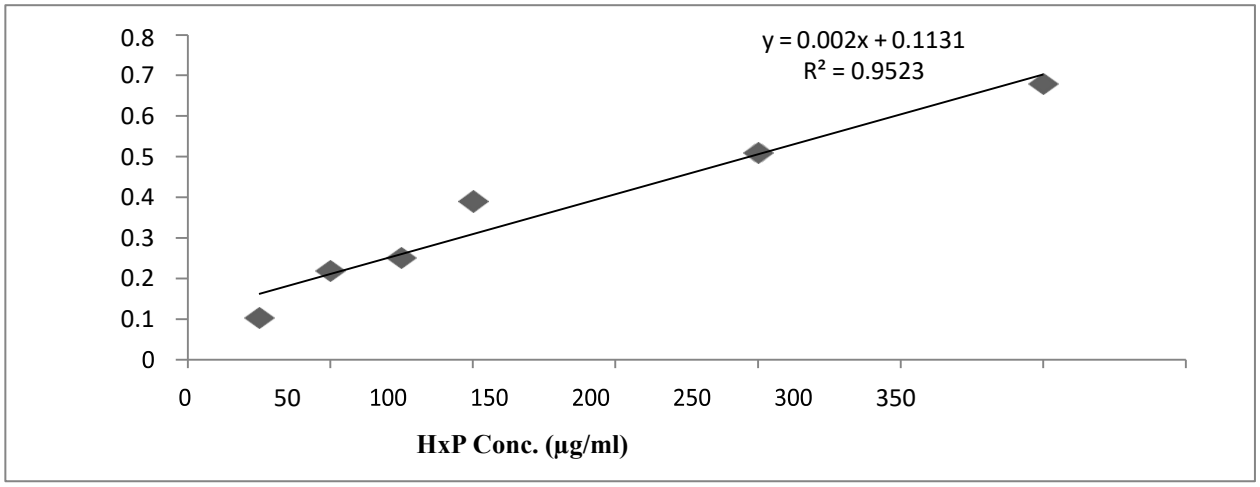
6. Oxidant solution: "The solution was prepared by diluting 1 volume of 7% chloramine-T solution in double distilled water with 4 volumes of citrate buffer (pH 6.0), making up a total volume of 10 ml".

7. Ehrlich's reagent: Mixed 17.6 g *p*-dimethylaminobenzaldehyde with 23.3 ml perchloric acid and made the volume to 1000 ml with *n*-propanol. The solution was freshly prepared.

8. HxP standard: 0.5 mg HxP was dissolved in 1.0 ml buffer to give the final conc. 500 µg/ml.

Table: 1.Parameters assessed in the present study.

Conc. (µg/ml)	HxP standard(ml)	Double distilled water (ml)	Sample taken (ml)	HCl (ml)	n-propanol (ml)	Oxidant(ml)	Ehrlich's reagent (ml)
25	0.05	0.95	0.1	0.4	1.0	0.5	1.0
50	0.10	0.90	0.1	0.4	1.0	0.5	1.0
75	0.15	0.85	0.1	0.4	1.0	0.5	1.0
100	0.20	0.80	0.1	0.4	1.0	0.5	1.0
200	0.40	0.60	0.1	0.4	1.0	0.5	1.0
300	0.60	0.40	0.1	0.4	1.0	0.5	1.0
The contents of the test tubes were mixed thoroughly and incubated at 60˚C for 21 min. Then, thetubes were cooled in ice bath for 60 min. The O.D. was measured at 562 nm against blank.							



Result and Discussion:

Bone mineral density (BMD)

"Table 27 and 28 indicate variations in the BMD of the lumbar vertebras and femoral bones as assessed by DEXA scans. The 4-month administration of PHT (35mg/kg) and SVP (100 and 300mg/kg) led, as compared to control groups, in a substantial reduction in BMD of lumbar vertebras and femoral mouse. Preventive therapy with RLX substantially recovered decreased BMD in both femoral and lumbar vertebrae after PHT and SVP (Table 25 & 26). While the lowered bMD was also recovered by preventative therapy of CVD, its effectiveness in the restitution of BMD in lumbar vertebras ($p<0.01$) following PHT and SVP, and femur after SVP, was less significant than that of RLX ($p<0.001$). Similar results were achieved in the administration for 1 month of post-pHT and PVS treatment of RLX and CVDD (therapy therapy) (Table 27 & 28) ".

Bone mineral content (BMC)

"Table 27 & 28 demonstrate the changes in the BMC of the lumbar vertebrae and femoral bones as assessed using DEXA scanning. For four months, PHT (35mg/kg) therapy caused the BMC of the femoral bones of mice to fall significantly compared to the control group. When therapy was increased by a further 1 month, the lumbar vertebrae also suffered a substantial decline in BMC. The lowered BMC after PHT, SVP and RLX preventive (not CVD) medication has been largely recovered. In the therapeutic group, however, the lowered BMC was dramatically re-established when both RLX and CVDD (therapeutic therapy) were supplied for one month after PHT. On the other hand, SVP therapy did not lead to substantial changes to BMC (Table 27 & 28) ".

Effect on biochemical markers of bone turnover

Alkaline phosphatase:

The PHT (35 mg/kg p.o.) and SVP (300 mg/kg p.o.) therapy considerably decrived ($p<0.001$), in contrast to control in both the preventative and therapeutic groups, the activities in the lumbal vertebra of bone alkaline phosphatase (ALP). While RLX, CVD or CVDD did not 97 exhibit a significant increase in lumbar ALP operation in relation to the control group ($p>0.05$), the AEDs-induced drop in bone ALP was considerably re-restored, with RLx being more successful than CVD/CVDD (Table 2 & 3) ".

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Tartrate Resistant acid phosphatase (TRAP)

"In lumbar vertebral mice treated with PHT (35 mg/kg) and SVP (300 mg/kg) both preventative and therapeutic groups, the activity of TRAP in both preventative and therapeutic groups was considerably increased (p<0,001). The high TRAP levels of both preventative and treatment groups were successfully controlled by RLX and CVD/CVDD with no significant impacts on the lumbar activities TRAP alone (Table 2 & 3) ".

Urinary Calcium (U-Ca)

"The urine excretions of calcium(U-CA) compared to the control rose considerably (p<0.001) with PHT (35 mg/kg) or SVP (300 mg/kg). Ca2+ urine excretion (p<0.001) has dramatically decreased preventive and therapeutic therapy with RLX. Similar CVD/CVDD effects were achieved, however the effects were weaker than RLX (p<0.05). (Tables 29 and 30) ".

Table 2: Effect of preventive and therapeutic treatment with raloxifene (RLX) on Phenytoin (PHT) induced changes in bone mineral density (BMD) and bone mineral concentration (BMC) of femur and lumbar vertebrae in mice.

Treatment	Groups	BMD		BMC	
		Femur	Lumbar Vertebrae	Femur	Lumbar Vertebrae
Therapeutic	Control (VEH)	0.092 ± 0.0018	0.107 ± 0.0022	0.026 ± 0.0021	0.023 ± 0.0021
	PHT	0.074 ± 0.0016***	0.088 ± 0.0009***	0.018 ± 0.0016*	0.016 ± 0.0021**
	PHT + RLX	0.095 ± 0.0028###	0.102 ± 0.0027###	0.033 ± 0.0021###	0.026 ± 0.0021##
	PHT + CVDD	0.098 ± 0.0034###	0.099 ± 0.0005##	0.026 ± 0.0021#	0.030 ± 0.000###
	RLX	0.098 ± 0.002###	0.111 ± 0.0010###	0.03 ± 0.000###	0.033 ± 0.002###
	CVDD	0.101 ± 0.0022###	0.104 ± 0.0033###	0.03 ± 0.000###	0.030 ± 0.000###
Preventive	PHT	0.074 ± 0.0016 ***	0.089 ± 0.0020***	0.018 ± 0.0016*	0.016 ± 0.0021
	PHT + RLX	0.098 ± 0.0018 ###	0.102 ± 0.0011###	0.03 ± 0.000##	0.04 ± 0.000***,###
	PHT + CVD	0.092 ± 0.0026 ###	0.1 ± 0.0020##	0.023 ± 0.0021	0.023 ± 0.0021
	RLX	0.099 ± 0.0005 ###	0.108 ± 0.0024###	0.033 ± 0.0021###	0.03 ± 0.000###
	CVD	0.096 ± 0.0049 ###	0.103 ± 0.0013###	0.026 ± 0.0021#	0.026 ± 0.0021##

Values are represented as mean ± SEM. VEH, Vehicle (Carboxymethylcellulose, 1ml/kg); PHT, Phenytoin (35 mg/kg); RLX, Raloxifene (15 mg/kg); CVD, Calcium Vitamin D (130 mg/kg + 65 IU); CVDD, CVD + VD [(130 mg/kg + 65 IU) + (195 IU)]; BMD, Bone Mineral Density; BMC, Bone Mineral Content. PHT was administered orally for a period of 4 months. Preventive treatments (RLX and CVD) were administered concurrently whereas therapeutic treatment (RLX and CVDD) was administered for 1 month after AEDs treatment. *p<0.05; ** p<0.01; *** p<0.001 when compared with vehicle control; # p<0.05; ##p<0.01; ### p<0.001 when compared with PHT.

Treatment	Groups	BMD		BMC	
		Femur	Lumbar	Femur	Lumbar
Therapeutic	Control (VEH)	0.092 ± 0.0018	0.107 ± 0.0022	0.026 ± 0.0021	0.023 ± 0.0021
	SVP	0.073 ± 0.0020***	0.084 ± 0.0007***	0.023 ± 0.0021	0.016 ± 0.0021
	SVP + RLX	0.089 ± 0.0029###	0.097 ± 0.001 ###	0.026 ± 0.0021	0.026 ± 0.0021#
	SVP + CVDD	0.093 ± 0.0020###	0.095 ± 0.0018##	0.026 ± 0.0021	0.026 ± 0.0021#
	RLX	0.098 ± 0.0020###	0.111 ± 0.001###	0.03 ± 0.000	0.033 ± 0.0021*,###
	CVDD	0.101 ± 0.0022###	0.104 ± 0.0033###	0.03 ± 0.000	0.03 ± 0.000###
Preventive	SVP	0.073 ± 0.002***	0.084 ± 0.0007***	0.023 ± 0.0021	0.016 ± 0.0021
	SVP + RLX	0.098 ± 0.0035###	0.102 ± 0.0012###	0.03 ± 0.0036	0.03 ± 0.0036##
	SVP + CVD	0.091 ± 0.0023##	0.096 ± 0.0045##	0.023 ± 0.0021	0.023 ± 0.0021
	RLX	0.099 ± 0.0005###	0.108 ± 0.0024###	0.033 ± 0.0021	0.03 ± 0.00##
	CVD	0.096 ± 0.0049###	0.103 ± 0.0013###	0.026 ± 0.0021	0.026 ± 0.0021#

Table 3: Effect of preventive and therapeutic treatment of raloxifene (RLX) on Sodium Valproate (SVP) induced changes in Bone mineral density (BMD) and bone mineral concentration (BMC) of Femur and Lumbar vertebrae in mice.

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Table 4: Effect of preventive and therapeutic treatment of Raloxifene (RLX) on Phenytoin (PHT) induced biochemical changes in lumbar vertebrae of mice.

Treatment	Groups	U-Ca^{2+} (mg Ca/mg of Cr)	ALP (µmol of PNP liberated/hr/µg of protein)	TRAP (µmol of PNP liberated/hr/µg of protein)	HxP (µg of HxP/mg of bone)
Therapeutic	Control (VEH)	0.066 ± 0.005	15.16 ± 1.06	0.53 ± 0.029	155.96 ± 2.93
	PHT	0.134 ± 0.005***	4.83 ± 0.44***	1.21 ± 0.065***	103.28 ± 1.45***
	PHT + RLX	0.069 ± 0.003###	12.87 ± 1.24###	0.64 ± 0.056 ###	148.35 ± 8.39 ###
	PHT + CVDD	0.102 ± 0.004*,#	10.55 ± 0.41##	0.75 ± 0.076 ##	139.64 ± 8.54 ##
	RLX	0.061 ± 0.01###	13.44 ± 1.48###	0.58 ± 0.082 ###	152.7 ± 4.42 ###
	CVDD	0.064 ± 0.01###	11.8 ± 0.91###	0.59 ± 0.086 ###	148.85 ± 7.77 ###
Preventive	PHT	0.121 ± 0.003**	5.83 ± 0.50***	0.95 ± 0.046 ***	115.28 ± 1.71***
	PHT + RLX	0.067 ± 0.003##	14.0 ± 0.94###	0.59 ± 0.03 ###	152.05 ± 3.54 ###
	PHT + CVD	0.078 ± 0.011#	12.09 ± 1.61##	0.63 ± 0.052 ###	149.38 ± 3.35 ###
	RLX	0.04 ± 0.012 ###	14.99 ± 0.99###	0.53 ± 0.032 ###	153.9 ± 4.99 ###
	CVD	0.055 ± 0.014###	13.01 ± 0.77###	0.54 ± 0.048 ###	151.62 ± 4.11 ###

Values are represented as mean ± SEM. VEH, Vehicle (Carboxymethylcellulose, 1ml/kg); PHT, Phenytoin (35 mg/kg); RLX, Raloxifene (15 mg/kg); CVD, Calcium Vitamin D (130 mg/kg + 65 IU); CVDD, CVD + VD [(130 mg/kg + 65 IU) + (195 IU)]; BMD, Bone Mineral Density; BMC, Bone Mineral Content. PHT was administered orally for a period of 4 months. Preventive treatments (RLX and CVD) were administered concurrently whereas therapeutic treatment (RLX and CVDD) was administered for 1 month after AEDs treatment.* P< 0.05; ** p<0.01; *** P<0.001, when compared with vehicle control; # p<0.05; ## p<0.01; ### P<0.001, when compared with PHT.

Table 5: Effect of preventive and therapeutic treatment of raloxifene (RLX) on Sodium Valproate (SVP) induced biochemical changes in lumbar vertebrae of mice.

Treatment	Groups/BTM	U-Ca^{2+} (mg Ca/mg of Cr)	ALP (µmol of PNP liberated/hr/µg of protein)	TRAP (µmol of PNP liberated/hr/µg of protein)	HxP (µg of HxP/mg of bone)
Therapeutic	Control (VEH)	0.066 ± 0.005	15.16 ± 1.06	0.53 ± 0.029	155.96 ± 2.93
	SVP	0.149 ± 0.012***	6.57 ± 0.39***	1.17 ± 0.128***	122.64 ± 3.45***
	SVP + RLX	0.077 ± 0.004###	12.66 ± 0.40##	0.60 ± 0.021 ###	147.21 ± 2.72 ##
	SVP + CVDD	0.103 ± 0.006#	11.61 ± 1.00#	0.69 ± 0.033 ##	142.94 ± 1.74 #
	RLX	0.061 ± 0.01###	13.44 ± 1.48###	0.58 ± 0.082 ###	152.7 ± 4.42 ###
	CVDD	0.064 ± 0.01###	11.80 ± 0.91##	0.59 ± 0.086 ###	148.85 ± 7.77 ##
Preventive	SVP	0.123 ± 0.007***	7.56 ± 0.757***	0.98 ± 0.037***	126.36 ± 3.34***
	SVP + RLX	0.064 ± 0.007###	13.57 ± 0.263###	0.56 ± 0.055 ###	149.87 ± 2.36 ###
	SVP + CVD	0.081 ± 0.006#	12.29 ± 0.798##	0.62 ± 0.04 ###	146.45 ± 2.31 ##
	RLX	0.04 ± 0.012###	14.99 ± 0.999###	0.53 ± 0.032 ###	153.9 ± 4.99 ###
	CVD	0.055 ± 0.014###	13.01 ± 0.774##	0.54 ± 0.048 ###	151.62 ± 4.11 ###

Values are represented as mean ± SEM. VEH, Vehicle (Carboxymethylcellulose, 1ml/kg); SVP, Sodium Valproate (300 mg/kg); RLX, Raloxifene (15 mg/kg); CVD, Calcium Vitamin D (130 mg/kg + 65 IU); CVDD, CVD + VD [(130 mg/kg + 65 IU) + (195 IU)]; BMD, Bone Mineral Density; BMC, Bone Mineral Content. SVP was administered orally for a period of 4 months. Preventive treatments (RLX and CVD) were administered concurrently whereas therapeutic treatment (RLX and CVDD) was administered for 1 month after AEDs treatment. *** $p<0.001$ When compared with vehicle control; # $p<0.05$; ## $p<0.01$; ### $p<0.001$ When compared with SVP.

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Discussion

Bone Mineral Density

Bone "deficiency, revealed in histology, was furthermore validated by measures of bone mineral density (BMD), which was recognised as a gold standard for diagnosing osteoporosis, using the Dual Energy X-ray absorptiometry (DEXA method). Both PHT and SVP have lowered BMD considerably which is consistent with preclinical and clinical research indicating BMD lowered after these AEDs (Boluk et al., 2004; Nissen-Meyer et al., 2007; Pack et al., 2008; Lee et al., 2010). PHT chronic therapy decreased the BMC, whereas SVP had no effect on BMC as it was on the BMD. Some early clinical results (Boluk et al. 2004; Go et al. 2001) were compatible with some but inconsistent with other SVPs, as noted in this investigation (Triantafyllou et al. 2010). An analysis of the strains-specific effects of valproate in animals published by Senn and co-workers (Senn et al.2010). Two sensitive (C3H/HeJ and balb/c) mouse strains and one strain (A/J) were discovered to withstand valproate-induced bone deficiencies. In our investigation, we are now reporting an additional strain (Swiss albino mouse) vulnerable to valproate-induced bone deficiencies. BMD was" not reduced after 4 times 114 by LTM (100 and 200 mg/kg)".
Treatment for "months this corresponds to the findings of Nissen-Meyer and colleagues on rats on BMD (Nissen-Meyer et al. 2007). However, the latter claim that LTM lowered the strength of the femoral neck's biomechanical" strength. Because LTM is mostly a trabecular bone, we examined if LTM causes modifications in the lumbar vertebrae (L2-L4), a second trabecular bone. However, "no alterations in our study in the lumbar vertebrae were visible and hence it might be plausible for LTM to have distinct effects on the femoral neck and lumbar locations that require research. Various reports were also available clinically. While the use of LTM monotherapy has not reporterally harmed bone strength and metabolism for one year of treatment and there is no apparent secondary effect on weight, quality and bone reshaping after two years of treatment (Koo et al., 2013), the latest report shows that after two years of treatment, LTM compromised BMD comparable to that of Oxcarbazepine (OXC) (Beniczky et al., 2012). Since the preceding study (Koo et al., 2013) reported the results of one single centre, conducted in a limited patient count and without taking account of the estimate of risk of fracture, there are potential adverse effects on the bone following LTM administered duely to increasing therapy or study duration in a broader population. However a recent research of young adult patients with epileptic disease also supports our findings on LTM that in patients switching to LTM, both lumbar spine and femoral BMD are somewhat greater compared with those of AEDs such as PHT injecting enzymes" (Phabphal et al., 2013).
The "preventive (RLX & CVD) and therapeutic (RLX & CVDD) therapy of femoral and lumbar vertebrae effectively recovered BMD after PHT and SVP (Table 28, 29). The efficacy of RLX in increasing lumbar BMD has been shown by multiples results of the raloxifene evaluation (MORE) where the increased BMD in the lumbar spine reduced the risk of vertebral disease by increasing the bone mass and bone mechanical strength of RLX in the prevention of vertebral fractures (Seeman et al., 2006). In addition, a recent antiepileptic medicine and osteoporosis prevention study (ADOPT) in epileptic men showed the favourable benefits of CVD supplementation on BMD at several locations, including lumbar spine (Lazzari et al., 2013), in accordance with our finding of the study. We have further studied its effect on biochemical markers exclusively of bone turnover in trabecular bone (lumba vertebras) as raloxifene has been reported more impacting trabecular"

Conclusion:

The undesirable Bony effects include the possible clinical effects of antiepileptic medicines (AEDs). Despite this, the impact of anti-osteoporotic therapy on AED bone loss is minimal. We assume that loss of oestrogen during AED medication might lead to deleterious consequences on the skin. AEDs inhibit the enzyme of human aromatase and increase oestrogen microsomal degradation. Estrogen deficit states have a recognised anti-osteoclastic feature, which diminish the deposition of a transforming growth factor-β (TGF-β3), a bone matrix protein. Therefore an attempt was made in comparison with calcium and vitamin D3 (CVD) supplementation to examine the effects of Raloxifen (RLX), a selective oestrogen receptor modulator, on AED-induction bone changes in the mice and to unravel the role of estradiol and TGF-β3 in mediate either "AEDs or raloxifene from a bone effect. In addition, raloxifene has also been explored on seizure and antiepileptic effectiveness of various AEDs.

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