

## Calcium and Phosphorus Utilization on Rats with Induced Cerebral Palsy

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**Abstract:** Cerebral palsy (CP) is characterized by motor impairment and can present with global physical and mental dysfunction. The aim of the present study was to investigate the utilization of calcium and phosphorus on rats with induced CP. Forty-five adult male albino rats weighing  $180 \pm 5$  gm were divided into groups. The 1st group ( $n=6$ ) was fed on the basal diet (-ve control). The 2nd-7th groups ( $n=39$ ) groups were injected with  $15 \mu\text{l}$  of the ethanol and  $50 \text{ mg/b.w}$  of sodium phenytoin for CP induction. The 2nd group was +ve control. Group 3 was fed on basal diet supplemented with vitamin D3 at level of  $600 \text{ IU kg/b.w}$ . Groups 4-5 were fed on diet supplemented with  $40 \text{ mg}$  &  $120 \text{ mg}$  of calcium (level 1 and 2), respectively. Group 6 was fed on diet supplemented with level 1 Ca ( $40 \text{ mg}$ ) with vitamin D supplementation, while group 7 was fed on diet supplemented with level 2 Ca with vitamin D addition. At the end of the experimental period (10 weeks), rats were scarified. Biochemical analysis of the tested parameters were determined. Results indicated that diet supplementation with calcium and/or with vitamin D separately or in combination significantly increased the final body weight and body weight gain %, as well as FER in all treated groups compared to the +ve control group. Liver functions, data revealed that feeding rats diets supplemented with Ca with / without vitamin D had improved liver functions by decreasing its elevated levels due to CP induction. Serum Ca and phosphorus (P) concentrations of CP rats were increased because of ingestion of diets supplemented with Ca and vitamin D in all tested groups. Consequently, Ca/P ratio of CP rats was improved. In addition, serum ALP and PTH concentrations of CP rats were improved. Data of our study revealed that diet supplementation with Ca and vitamin D at the tested levels improved bone mineral density for CP rats. The best improvement was seen when diet contains the highest level of Ca in addition to vitamin D. However, bone minerals content of the +ve control group showed significant reduction. It could be concluded from our study that ingestion of diets supplemented with Ca and vitamin D had beneficial effects on utilization of Ca and P and its ratio of CP rats. Thus, results of the current study recommend that vitamin D and calcium supplementation at the tested levels could be worthy treating for CP patients.

**Keywords:** cerebral palsy, calcium, phosphorus, sodium phenytoin, bone mineral content, calcium/phosphorus ratio

### 1. INTRODUCTION

Cerebral palsy (CP) is a group of disorders of the development of movement and posture, causing activity limitation that is attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. This group of syndromes ranges in severity and is the result of a variety of etiologies occurring in the prenatal, perinatal or postnatal period. CP is the most common form of chronic motor disability in children. Other causes of disability in children include prolonged immobilization, nutritional factors, pubertal disorder and chronic use of anticonvulsants. All these factors may be related to impairment of normal bone development. Low calcium intake is common in CP patients and may contribute to poor

mineralization of bone matrix. Consequently, children with severe CP are at risk for developing low bone mineral density (BMD) and low-impact fractures (Nikam *et al.*, 2019).

Vitamin D is essential for normal skeletal development, mineralization, growth and for maintenance of skeletal health throughout life, and those assessed vitamin D status and optimal means of improving status in these patient populations Vitamin D promotes calcium absorption from the intestine. Adequate vitamin D concentrations prevent bone loss and decrease fracture risk in the elderly (Arslan *et al.*, 2018).

Calcium is essential for normal bone health at all ages. During childhood, calcium is needed for skeletal growth and in adulthood for maintenance of bone mass and content.

Inadequate calcium intake results in decreased calcium absorption in the intestine, low levels of ionized calcium, and increased secretion of PTH (Peacock, 2010).

Many studies report that high P intake is harmful to bone health in subjects whose dietary Ca: P ratio is extremely low. However, there is strong evidence that high P intake has no negative impact on Ca balance in normal subjects with appropriate dietary Ca and P intakes (Lee and Cho, 2015).

Therefore, our study was conducted to investigate the effect of dietary calcium and vitamin D supplementation on calcium and phosphorus utilization on rats with induced cerebral palsy.

## 2. MATERIALS AND METHODS

### 2.1. MATERIALS

Casein, all vitamins, calcium phosphate, minerals, ethanol, L.cystine and choline bitartrate were obtained from El-Gomhoria Company, Cairo, Egypt. Capsules of cholecalciferol (vitamin D3) at concentration of 1000 IU and sodium phenytoin (PHT) at concentration of 50 mg were obtained from Delmar and Attallah Pharmacy, Cairo, Egypt.

Starch, corn oil, and sucrose were obtained from the local market Kits for blood analysis were purchased from local distributor (Sigma Chemical), Cairo, Egypt.

Animals: A total number of forty five adult male Albino rats (Sprague -Dawley strain) weighing  $180 \pm 5$  gm, were purchased from the Animal House of Helwan Farm, Helwan, Egypt.

### 2.2. METHODS

#### 2.2.1. Induction of Cerebral Palsy (CP) in Rats

The left pyramidal tract of adult Sprague-Dawley rats was placed on sterile operation table under intraperitoneal anesthesia. Based on the rat brain stere-otaxic map, a 1-mm hole was made 10 mm posterior to bregma and 0.8 mm left of sagittal suture. A micro syringe was inserted perpendicularly to the surface of the brain to a depth of 9.7 mm, and 15  $\mu$ L of ethanol was slowly injected to establish a rat model of spastic cerebral palsy as stated by Yadong *et al.*, (2013). Rats with CP then received phenytoin tablets in concentration of 50 mg at the level of 20 mg/ml/kg b.w to alleviate the symptoms of CP.

#### 2.2.2. Diet Composition and Experimental Animal Design

The basal diet was formulated according to AIN-93M diet (Reeves, *et al.*, 1993). Calcium

carbonate and phenytoin were dissolved in distilled water and received by oral tube according to body weight of rats. Capsules of cholecalciferol (vitamin D3) was dissolved in corn oil and administrated with oral tube according to body weight of rats.

Forty five adult male Albino rats were housed in well- aerated cages under hygienic conditions and were fed on the basal diet for one week for adaptation. After this period, rats were divided into groups as follows: The first main group (n= 6) was fed on basal diet and served as a negative control group (-ve). The rest of the rats (39 rats) were injected with 15 $\mu$ L of ethanol for induction of CP. Three rats were scarified and the femur bone was taken to insure the induction of the CP using DEXA examination. Then rats were divided into six groups (6 rats each) as follows:

Group (1): Normal rats were fed on basal diet and served as a negative control group (-ve).

Group (2): Rats with CP were fed on basal diet (+ve control).

Group (3): Rats with CP were fed on basal diet and given daily vitamin D3 (600 IU/ kg. b. w) orally.

Group (4): Rats with CP were fed on basal diet supplemented with 40mg calcium phosphate (level1).

Group (5): Rats with CP were fed on basal diet supplemented with 120 mg calcium phosphate (level 2).

Group (6): Rats with CP were fed on basal diet supplemented with 40 mg calcium phosphate (level1) and Vitamin D.

Group (7): Rats with CP were fed on daily basal diet supplemented with 120 mg calcium phosphate (level2) and Vitamin D.

At the end of the experimental period (10 weeks), rats were anesthetized with diethyl ether after fasting for 12 h and blood samples were taken. Then serum was carefully separated into vacuum tubes and kept frozen until biochemical analysis. Femur bones were removed from each rat for DEXA analysis.

#### 2.2.3. Biological Evaluation

Biological Evaluation: feed intake (FI), feed efficiency ratio (FER) and body weight gain percent (BWG%) were determined according to Hemdanand Abdulmaguid, (2018) using the following equation:

$$BWG\% = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

$$FER = \text{Body weight gain (g)} / \text{Feed intake (g)}$$

2.2.4. Biochemical Analysis

Serum calcium content was determined according to (Gosling, 1986). Serum phosphorus content was determined according to (Fogg and Wilkinson, 1958). Serum alkaline phosphates was determined by immunosorbent assay according to the method described by Roy, (1970). Alanine aminotransferase (ALT) was determined by immunosorbent assay according to the method described by Thefeld, (1974). Aspartate aminotransferase (AST) was determined by immunosorbent assay according to the method described by Thefeld, (1974). Serum parathyroid hormone was determined by immunosorbent assay according to the method described by Henderson et al., (1990).

2.2.5. Bone Mineral Content

The density of bone in the right femur was determined by DEXA UNITE (LUNAR OX IQ 4716) according to the method of EL- Adawy, (1986)

2.2.6. Statistical Analysis

The Results were expressed as means± standard error (SE). The obtained results were analyzed according to SPSS program, Version (20). Analysis of variance (ANOVA) test was used to compare results among groups and P≤ 0.05 was considered significant (Snedecor and Cochran, 1980).

**Table1.** Effect of diets supplemented with Ca and Vitamin D on body weight, feed intake(FI) and feed efficacy ratio(FER) of Cerebral Palsy rats

Groups	Parameters				
	IBW (g)	FBW (g)	BWG%	FI (g/day)	FER
Control (-Ve)	183±1.96 <sup>a</sup>	262±2.30 <sup>a</sup>	43±1.76 <sup>a</sup>	19.10	0.05±0.001 <sup>a</sup>
Control (+Ve)	182±2.03 <sup>a</sup>	192.±1.71 <sup>e</sup>	5±0.48 <sup>e</sup>	11.00	0.01±0.0009 <sup>d</sup>
Vitamin D	180±1.02 <sup>a</sup>	205±2.08 <sup>d</sup>	13±1.04 <sup>d</sup>	13.70	0.02±0.001 <sup>c</sup>
Level 1 Ca	184±1.24 <sup>a</sup>	240±5.41 <sup>c</sup>	29±2.28 <sup>c</sup>	14.00	0.05±0.004 <sup>ab</sup>
Level 2 Ca	185±1.82 <sup>a</sup>	236±1.52 <sup>c</sup>	27±1.46 <sup>c</sup>	15.00	0.04±0.002 <sup>b</sup>
Level (1) Ca +Vt.D	183±1.96 <sup>a</sup>	238±2.88 <sup>c</sup>	30±2.71 <sup>c</sup>	15.30	0.04±0.003 <sup>ab</sup>
Level (2) Ca + Vt.D	183±2.70 <sup>a</sup>	249±0.75 <sup>b</sup>	35±2.23 <sup>b</sup>	15.50	0.05±0.002 <sup>a</sup>

\*Values are expressed as means ± SE.

\*Values at the same column with different letters are significantly different at P<0.05.

\*IBW= Initial body weight, FBW= final body weight, BWG%=Body weight gain percent, FI= feed intake, FER=feed efficiency ratio.

Regarding to liver functions in **Table (2)**, there were significant (P<0.05) increase in serum ALT and AST levels of the positive control group (rats with CP) compared with the negative control group (normal rats). Feeding rats diets supplemented with vitamin D (group3) or Ca at the two different levels separately or in combination with vitamin D, caused significant (P<0.05) decrease in serum ALT activity, compared to the positive control group. It was clear that, there was no significant difference in

3. RESULTS

Results illustrated in **Table (1)** show the effect of diets supplemented with calcium and vitamin D on body weight, feed intake and feed efficiency ratio of cerebral palsy rats. There were no significant changes in the initial body weight of all groups of rats. The final body weight (FBW) and body weight gain percent (BWG%) were significantly (P< 0.05) lowered in the positive control group (CP rats) compared to the control one. Diet supplementation with calcium and/or with vitamin D separately or in combination significantly increased the final body weight and body weight gain percentage, compared to the positive control group. It was also observed that the highest final body weight and body weight gain % were recorded at the group that fed on high calcium level (120mg) with vitamin D (group 7). Moreover, the mean feed intake was increased at all different treated groups compared to the positive control group. It was also observed that there were no significant differences in FER among the groups treated with calcium alone at 120 mg and the groups fed on calcium (40 mg or 120 mg) in addition to vitamin D as compared to the negative control group. In conclusion, body weight gain of CP rats was increased as a result of ingestion of diets supplemented with calcium and Vitamin D in all tested groups.

serum ALT concentration among all the tested groups. Moreover, there was no significant difference in serum AST level among the groups fed on Ca at the level of 40 mg or 120 mg, also between the groups fed on Ca at the two tested levels in addition to vitamin D. There was no significant change in serum AST among the groups fed on Ca at the two tested levels with vitamin D and the negative control group. The highest improvements for liver functions were observed at the group of rats that fed on Ca at

120mg with vitamin D. Thus, our study showed that ingestion of calcium and vitamin D

improved serum liver functions concentration of cerebral palsy rats.

**Table2.** Effect of diets supplemented with Ca and Vitamin Don serum liver functions in rats with Cerebral Palsy

Parameters	ALT (U/L)	AST(U /L)
<b>Group1(-VeControl)</b>	18.80±1.12 <sup>c</sup>	33.93±1.57 <sup>d</sup>
<b>Group 2 (+VeControl)</b>	60.26±4.56 <sup>a</sup>	70.40±3.03 <sup>a</sup>
<b>Group 3(Vitamin D)</b>	44.76±4.72 <sup>b</sup>	58.66±3.17 <sup>b</sup>
<b>Group 4 (Level 1 Ca)</b>	45.33±2.02 <sup>b</sup>	52.26±5.31 <sup>cb</sup>
<b>Group 5 (Level 2 Ca)</b>	41.90±2.26 <sup>b</sup>	48.00±3.76 <sup>cb</sup>
<b>Group6 (Level (1) Ca +Vt.D)</b>	44.00±1.73 <sup>b</sup>	43.23±2.42 <sup>cd</sup>
<b>Group 7 (Level (2) Ca + Vt.D)</b>	38.73±3.12 <sup>b</sup>	41.13±4.49 <sup>cd</sup>

\*Values are expressed as means ± SE.

\*Values at the same column with different letters are significantly different at P<0.05.

Effect of diets supplemented with calcium and vitamin Don serum calcium, phosphorus and the ratio of calcium to phosphorus of cerebral palsy rats is shown in **Table (3)**. Positive control group (CP rats) had significant decrease (P<0.05) in the concentrations of serum calcium compared to the negative control group (normal rats) with mean values of 4±0.64mg /dl vs 11±1.02 mg /dl, respectively. When animals were fed on diets supplemented with vitamin D and/or calcium at the two different tested levels separately or in combination with vitamin D significantly increased the concentration of

serum calcium, phosphorus and calcium to phosphorus ratio compared to the positive control group. There was no significant change in calcium and phosphorus levels between the groups fed on calcium at the two different levels ( 40 mg or 120 mg), also between the groups fed on calcium at the two different levels in addition to vitamin D. In conclusion, the serum calcium and phosphorus concentrations of CP rats were increased as a result of ingestion of diets supplemented with calcium and vitamin Din all tested groups. Consequently, calcium to phosphorus ratio of CP rats was improved.

**Table3.** Effect of diets supplemented with Ca and vitamin D on serum calcium and phosphorus in rats with cerebral palsy

Parameters	Calcium (mg /dl)	Phosphorus (mg /dl)	Ca :P ratio
<b>Group1(-Ve Control)</b>	11±1.02 <sup>a</sup>	6±0.29 <sup>a</sup>	1.8
<b>Group 2 (+Ve Control)</b>	4±0.64 <sup>d</sup>	3±0.55 <sup>d</sup>	1.3
<b>Group 3(Vitamin D)</b>	7±0.12 <sup>c</sup>	4±0.21 <sup>cb</sup>	1.7
<b>Group 4 (Level 1 Ca)</b>	8±0.19 <sup>cb</sup>	4±0.07 <sup>c</sup>	2.0
<b>Group 5 (Level 2 Ca)</b>	8±0.11 <sup>cb</sup>	4±0.20 <sup>cb</sup>	2.0
<b>Group6 (Level (1) Ca +Vt.D)</b>	9±0.55 <sup>b</sup>	5±0.25 <sup>b</sup>	1.8
<b>Group 7 (Level (2) Ca + Vt.D)</b>	9±0.21 <sup>b</sup>	5±0.26 <sup>b</sup>	1.8

\*Values are expressed as means ± SE.

\*Values at the same column with different letters are significantly different at P<0.05.

The results in **Table (4)** illustrated the effect of diets supplemented with calcium and vitamin D on serum alkaline phosphatase and parathyroid hormone of CP rats. Cerebral palsy rats had a significant (P<0.05) increase in serum alkaline phosphatase and parathyroid hormone (due to CP induction) compared to the negative control ones. When CP rats were fed on basal diet and given orally vitamin D (group 3) showed significant reduction in alkaline phosphatase and parathyroid hormone concentrations in serum with mean values of 1281.00±21.00U/L and 111.90±2.27 U/L, respectively compared to the CP group( group 2). Ingestion of calcium at low(40 mg) or high (120mg) level separately or

in combination with vitamin D, caused more significant decrease (P<0.05) in serum alkaline phosphatase and parathyroid hormone compared to the positive control group.

In general there were significant decrease in serum alkaline phosphatase and parathyroid hormone among the groups given Vitamin D and the group given calcium at the two different levels. The most improvement in serum ALP and PTH were recorded in the group given calcium at 120mg in addition to vitamin D. Thus, our study showed that ingestion of diets supplemented with calcium and vitamin D improved serum ALP and PTH concentration of cerebral palsy rats.



**Table4.** Effect of diets supplemented with Ca and vitamin D on serum ALP and PTH in rats with cerebral palsy

Parameters	Alkaline Phosphatase (U/L)	Parathyroid Hormone (U/L)
<b>Group1(-Ve Control)</b>	609.33±18.41 <sup>fe</sup>	44.83±5.91 <sup>e</sup>
<b>Group 2 (+Ve Control)</b>	1611.33±26.73 <sup>a</sup>	148.56±3.50 <sup>a</sup>
<b>Group 3 (Vitamin D)</b>	1281.00±21.00 <sup>b</sup>	111.90±2.27 <sup>b</sup>
<b>Group 4 (Level 1 Ca)</b>	1139.00±23.57 <sup>c</sup>	99.00±1.47 <sup>c</sup>
<b>Group 5 (Level 2 Ca)</b>	925.40±34.30 <sup>d</sup>	77.36±3.45 <sup>d</sup>
<b>Group6 (Level (1) Ca +Vt.D)</b>	681.46±33.19 <sup>e</sup>	67.33±4.22 <sup>d</sup>
<b>Group 7 (Level (2) Ca + Vt.D)</b>	553.53±75.38 <sup>f</sup>	49.13±3.69 <sup>e</sup>

\*Values are expressed as means ± SE

\*Values at the same column with different letters are significantly different at P<0.05.

Effect of diet supplemented with Ca and vitamin D on Bone mineral density and bone mineral content in rats with cerebral palsy shown in Table (5) as well as dual energy X-ray Scan of cerebral palsy rats is illustrated in photos 1-7. The mean bone mineral density of the positive control group was significantly (P<0.05) decreased, compared to the negative control rats (normal group). The diet supplementation with vitamin D or calcium at the two different levels separately or in combination with vitamin D showed significantly (P<0.05) increased the mean value of bone mineral density, compared to the positive control group. There was no significant change in bone mineral density between the groups given calcium in the diet either at low or high level. However, there was a significant increase in bone mineral density for rats given calcium at high level with vitamin D

addition, compared to either the rats given calcium at the low level with of vitamin D or rats given vitamin D alone. The highest increase in the bone mineral density was observed for the groups given calcium at high level with vitamin D supplementation.

Regarding to bone mineral content, it was seen that there were no significant differences among all the treated groups compared to the negative and the positive control ones. Also, no significant change in the mean value of bone mineral content for the positive control group compare to the negative control group. It could be concluded that diet supplementation with calcium and vitamin D at the tested levels improved the bone mineral density for CP rats. The best improvement was seen when diet contains the highest level of Ca in addition to vitamin D.

**Table5.** Effect of diet supplemented with Ca and vitamin D on Bone Mineral Density and Bone Mineral Content in rats with cerebral palsy

Parameters	Bone Minerals Density (g/Cm2)	Bone Mineral Content (gm /cm2)
<b>Group1(-Ve Control)</b>	0.08±0.003 <sup>d</sup>	0.35±0.10 <sup>a</sup>
<b>Group 2 (+Ve Control)</b>	0.07±0.001 <sup>e</sup>	0.35±0.02 <sup>a</sup>
<b>Group 3 (Vitamin D)</b>	0.09±0.001 <sup>cd</sup>	0.35±0.06 <sup>a</sup>
<b>Group 4 (Level 1 Ca)</b>	0.10±0.002 <sup>c</sup>	0.26±0.04 <sup>a</sup>
<b>Group 5 (Level 2 Ca)</b>	0.10±0.006 <sup>b</sup>	0.27±0.07 <sup>a</sup>
<b>Group 6 (Level (1) Ca +Vt.D)</b>	0.10±0.003 <sup>b</sup>	0.27±0.00 <sup>a</sup>
<b>Group 7 (Level (2) Ca + Vt.D)</b>	0.11±0.002 <sup>a</sup>	0.33±0.06 <sup>a</sup>

\*Values are expressed as means ± SE

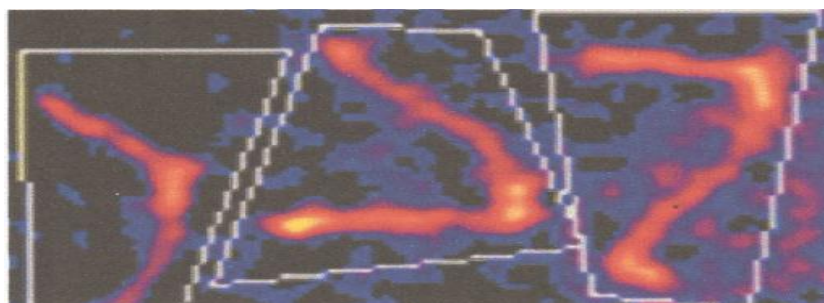
\*Values at the same column with different letters are significantly different at P<0.05.



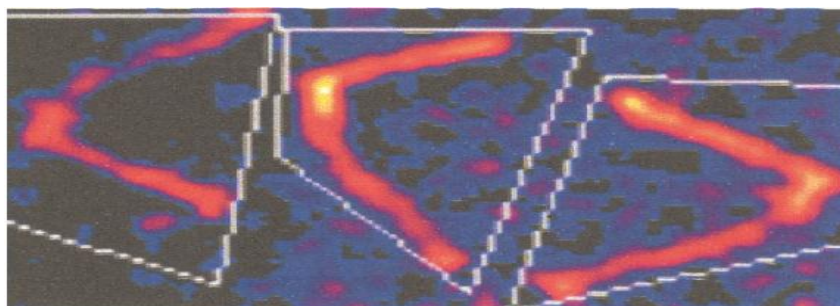
**Photo1.** Dual energy X-ray Scan of cerebral palsy rats from the negative control group (group1)



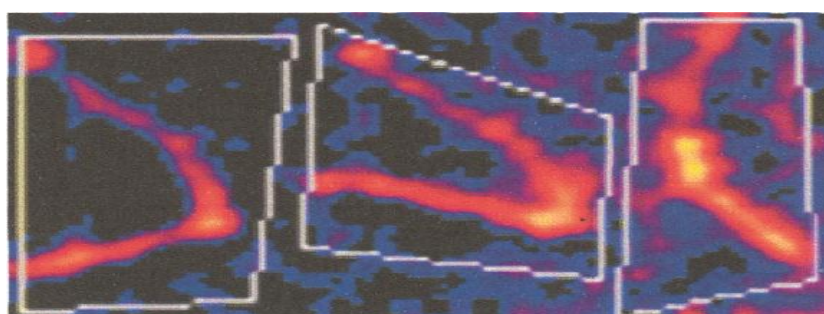
**Photo2.** *Dual energy X-ray Scan of cerebral palsy rats from the positive control group (group 2)*



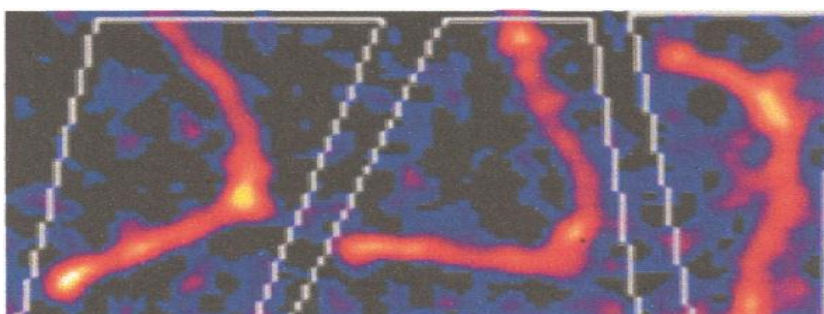
**Photo3.** *Dual energy X-ray Scan of cerebral palsy rats fed on basal diet supplemented with vitamin D (group3)*



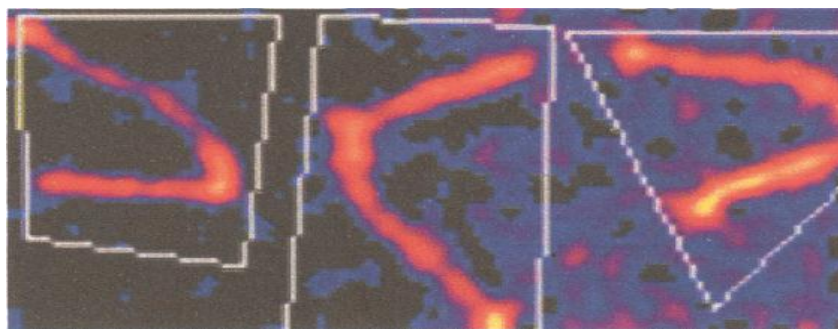
**Photo4.** *Dual energy X-ray Scan of cerebral palsy rats fed on basal diet supplemented with level 1 Ca (group 4)*



**Photo5.** *Dual energy X-ray Scan of cerebral palsy rats fed on basal diet supplemented with level 2 Ca (group5)*



**Photo6.** *Dual energy X-ray Scan of cerebral palsy rats fed on basal diet supplemented with level 1 Ca with Vt. D (group 6)*



**Photo7.** Dual energy X-ray Scan of cerebral palsy rats fed on basal diet supplemented with level 2 Ca with Vt.D (group 7)

#### 4. DISCUSSION

The cerebral palsy brain lesion can occur, to neonatal or at a later stage in a child's life. The true proportion of children affected antenatal is not known however, indicators of causes such as genetic predisposition prior to conception or infections encountered during pregnancy amongst others can be determined. For those infants who suffered their lesion or insult perinatally, only a fraction is associated with poor obstetric care during birth. The precise figure is unknown, as diagnosis cannot be made until developmental milestones are missed, however by the age of 5 years, 0.2% of all births will have cerebral palsy (Blair *et al.*, 2018).

Children with CP are at risk for developing low bone quality and low-impact fractures. Important risk factors compromising bone health in this group are immobility, malnutrition and medication use for example antiepileptic drugs (Papadelis *et al.*, 2018). Children with severe CP develop over the course of their lives clinically significant osteopenia. Unlike elderly adults, this is not primarily from true losses in bone mineral, but from a rate of growth in bone mineral that is diminished relative to healthy children (Toopchizadeh, *et al.*, 2019).

Results of the current study indicated that the highest improvements for liver functions (ALT, AST, and ALP) were observed with the group of rats that fed on diet supplemented with Ca at 120mg in addition to vitamin D. This result is confirmed by the findings of Moghadam *et al.*, (2018) who found that significant elevations in serum alkaline phosphatase levels were evident in all patient groups who are receiving phenytoin without giving any supplements. In 2018, it has been shown by Nair that concomitant use of antiepileptic drugs (AEDs) that induce microsomal P450 enzymes (e.g., phenytoin and phenobarbital) may enhance the production of a toxic metabolite, and hence the greater risk of hepatotoxicity with polypharmacy. On the other hand, Okwakpam,

*et al.*, (2018) suggested that there is no value in the routine performance of liver function tests in patients with epilepsy. However, such patients should be informed of the symptoms of hepatic dysfunction and asked to report for liver function tests should they have such symptoms.

The results of the current study indicated that diet supplemented with vitamin D and calcium at the highest-level increased serum calcium, phosphate concentrations, while the mean values of serum alkaline phosphatase level was decreased in the cerebral palsy (CP) rats. This result is confirmed by the findings of Mughal, (2018) who found that the mean serum calcium (Ca) and phosphate (P) levels were increased (from 2.17 to 2.35 mmol/L for Ca and from 1.13 to 1.66 mmol /L for P). However, the mean total alkaline phosphatase level was decreased from 1123 to 423 U/L among children and young adults with quadriplegic cerebral palsy. Some other studies (Choi and Seo, 2013, Lu *et al.*, 2013 and Papadelis *et al.*, 2014) confirmed that calcium and vitamin D are critical regulators of bone remodeling, promoting weight-bearing activity are also preventive measures that required in the care for children and adults with CP.

It has been shown in the current study that there was significant reduction in parathyroid hormone activities among rat groups given diets supplemented with vitamin D only, and the groups of rats were given diets supplemented with calcium at the two different levels in addition to vitamin D. The most improvement in serum PTH concentration was recorded in the group given calcium at 120mg with vitamin D. These findings could be due to that PTH regulates blood calcium levels by releasing calcium from bone, and by stimulating calcium reabsorption by the kidney. Parathyroid hormone also stimulates the renal synthesis of 1,25 (OH)<sub>2</sub>D, thus increasing intestinal calcium absorption as stated by Kilpinen-Loisa *et al.*, (2010).



**Sözenet et al., (2017)** found that inability to absorb adequate amounts of calcium for optimal bone health, as well as an increased susceptibility of falling, impaired muscle strength, and increased rates of bone loss can increase the risk of fracture. **Kandhro et al., (2019)** reported that lower levels of vitamin D are independently associated with an increased risk of falling in the elderly. In fact, supplementation with vitamin D has been shown to improve musculoskeletal function and reduce the risk of falling in elderly women. **Stockton, et al., (2011)** confirmed that by stating that human muscle contains vitamin D receptors that may lead to increasing muscle strength and improving stability. Also, another study by **Kamberi et al., (2018)** found that the combination of 700 units/day of vitamin D and 500 mg of calcium (obtained through supplementation in addition to diet) was shown to reduce falls by as much as 65% over three years in less active women over the age of 65. **LeBoff et al., (2019)** found that over 18 months, a trial of elderly french women found that the number of hip fractures was reduced by 43% and no vertebral fractures was reduced by 32% among women treated with calcium (1200 mg/day) and vitamin D (800 units/day) than among those who received placebo. **Sandhu and Hampson, (2011)** confirm that the best effect was seen with minimum doses of 1200 mg of calcium and 800 units of vitamin D daily and calcium, or calcium in combination with vitamin D supplementation, was effective in the preventive treatment of osteoporosis.

Concerning bone mineral density, the results of the current study indicated that diets supplementation with vitamin D and calcium at the two studied levels significantly improved the mean level of bone mineral density (BMD) in CP rats. Some studies revealed that fractures correlated with low body mass index (BMI), stiff joints, poor balance, and violent seizures (**Snyder and Anderson, 2016**). **Leijten, et al., (2019)** confirms that low BMD is a serious problem in children with severe CP, significant determinants of low BMD were limited ambulation feeding difficulties, previous fracture, anticonvulsant use, and lower fat mass (measured at the triceps skinfold). Other studies by **Kilpinen-Loisa et al., (2010)** and **Finbraten et al., (2015)** suggested that most children with motor disability have low BMD and subsequently increased susceptibility to fractures. These results were in agreement with

the findings in the present study. **Mohammad et al., (2018)** indicated that supplementation with vitamin D and calcium at the two studied levels significantly improved the mean level of bone mineral content (BMC) in CP rats.

It could be concluded from our study that ingestion of diets supplemented with calcium and vitamin D improved liver functions of cerebral palsy rats. Also, had beneficial effects on utilization of calcium and phosphorus and consequently, calcium to phosphorus ratio by increasing its concentrations in serum of CP rats. Thus, results of the current study recommend that vitamin D and calcium supplementation at the tested levels could be worthy treating for cerebral palsy patients.

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