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EVALUATION OF SOME FORTIFIED CALCIUM BASED FOOD FOR OSTEOPOROSIS PREVENTION

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ABSTRACT

This study was carried out to demonstrate the effect of feeding the osteoporotic rats with different diet formulas fortified with different calcium sources. The osteoporotic rats which were produced from feeding the ovariectomized female albino rats on low calcium diet for 30 days, were fed on different diet formulas including basal diet, basal diet and lentil soup or basal diet and vegetable soup which were fortified each with different calcium sources-egg shell, oyster shell or calcium citrate-for 45 days-All osteoporotic treated groups recovered their serum protein, alkaline phosphatase activity, total calcium, ionized calcium and phosphorus at the end of experimental period. The highest recovery was observed in the groups which received oyster shell as calcium source in the different formulas, especially in the presence of soups. While, calcium citrate comes in the second place and egg shell comes in the last place with slight differences between the three calcium sources .Therefore, it can be concluded that consuming egg shell as calcium source is a cheap and good source of utilizable calcium.

INTRODUCTION

Calcium is the most abundant mineral in the body. More than 99% of calcium is located in bone, where it plays an important role in their structure and strength, while a very small proportion of body calcium plays vital part in regulating critical functions including nerve impulses, muscle contractions and the activities of enzymes So critical is calcium's role in metabolic regulation that its concentration in the blood needs to be maintained within a constant range. If insufficient calcium is obtained from the diet for this purpose, the bones act as a store of calcium from which the element can be withdrawn to keep the blood level constant **(Gurr, 1999)**.

Osteoporotic is a disease in which loss of bone including calcium, results in weakening of the bone structure which increase the likelihood of fractures occurring. Osteoporosis and related fractures are a major cause of morbidity and mortality in the aging population **(Prince, 1997)**.

The important role of calcium and vitamin D, in decreasing bone loss and osteoporotic fractures in elder's men and women, is widely recognized. Increasing intake of calcium and vitamin D in later years can slow the rate of bone loss and reduce risk of osteoporotic fractures **(Dawson *et al* 1997)**. Several studies indicate that recommended calcium intake of 1200-1500mg/day **(American Medical Association, 1997)** is necessary to protect older adults bone health. **Chapuy and Arolt (1992)** mentioned that women who increased their calcium intake to 1700mg/day and consumed on additional vitamin D_3 for 18 months reduced the rate of hip fractures by 41% and other vertebral

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fractures by 30%. However, it is difficult for many people to consume diets suppling the recommended calcium intake (1200-1500mg/day), which are needed to achieve optimal bone density and protect against bone loss.

Knowing that the usual intakes of dietary calcium are below recommended amounts for most elderly persons, increased use of traditional calcium rich foods appears imperative to increase dietary calcium intakes.

The bioavailability of calcium from different sources can vary significantly (**Karkkainen *et al* 1997**). Little information are available about the relative bioavailability of calcium from the different commercial products, especially for elderly people, who may have compromised ability to absorb different forms of calcium (**Pattanaunkgul *et al* 2000**).

Thus this study was carried out to evaluate some food products enriched with different calcium sources including egg shell as natural locally source, oyster shell as imported natural source and calcium citrate. The calcium enriched products were lentil and vegetable soups.

MATERIALS AND METHODS

Preparation of soups

All the raw material used were purchased from the market of Giza governorate. Two types of soup were prepared using a mixture of dried materials, the first was lentil soup (Lentil 70.35%, Carrot 15.07%, Tomatoes powder 0.61%, Onion 7.02%, garlic 3.11%, Celery 3.29% and Spices 0.55%) and the other was vegetable soup (Green bean 45.93%, Carrot 32.79%, Tomatoes powder 1.63%, Onion 16.39%, Garlic 0.98%, Celery 0.65%, Parsley 1.63%). All the vegetable used were dried under vacuum before the preparation of soup at 60°C for 12-20h. Each soup mixture was divided into 4 portions. The first portion used as control and a sources of calcium (egg shell containing calcium 39.5%, oyster shell containing calcium 39% or calcium citrate) were added to the other portions. The soup mixtures were packed in polypropylene bags, stored at 4°C.

The diet composition

The composition of basal diets was casein 10%, corn oil 10%, salt mixture 4%, vitamins mixture 1%, and cellulose 5%, Sugar 30% and starch 40%. The composition of the salt mixture and vitamins mixture requirements was given according to (**AOAC 1995**).

Feeding experiment

One hundred twelve female albino rats with a body weight ranging from 90-110g were used in this study. Eight rats-initial group-were randomly chosen, then weighed, blood samples were withdrawn from retro bulbar venous plexus of each rat according to the procedure of **Shermer (1967)**. Serum was separated and the serum biochemical parameters were estimated, then rats were sacrificed, and organs including liver, kidney, heart, lung and spleen were excised and weighed.

The remaining 104 rats were ovariectomized and were fed on low calcium basal diet containing 0.01% calcium for 30 days to produce experimental osteoporotic model. At the end of the feeding period eight rats were randomly chosen-osteoporotic group-then blood samples were taken, and serum was separated and used for the biochemical analyses. Rats were weighed, sacrificed and organs were excised and weighed.

The remaining 96 rats were randomly divided into 12 groups each of eight rats as fallows:

Control^{+}	: Rats were fed on basal diet.
Group I	: Rats were fed on basal diet and egg shell
Group II	: Rats were fed on basal diet and oyster shell
Group III	: Rats were fed on basal diet and calcium citrate.
Control^{++}	: Rats were fed on basal diet and lentil soup.
Group IV	: Rats were fed on basal diet and lentil soup fortified with egg shell as calcium source
Group V	: Rats were fed on basal diet and lentil soup fortified with oyster shell as calcium source
Group VI	: Rats were fed on basal diet and lentil soup fortified with calcium citrate as calcium source
Control^{+++}	: Rats were fed on basal diet and vegetable soup
Group VII	: Rats were fed on basal diet and vegetable soup fortified with egg shell as calcium source
Group VIII	: Rats were fed on basal diet and vegetable soup fortified with oyster shell as calcium source.
Group IX	: Rats were fed on basal diet and vegetable soup fortified with calcium citrate as calcium source.

The content of soup in the whole diet in all groups was 20%, and all fortified diet formulas containing 7.5g calcium/kg diet. Feeding duration was 45 days. Rats were kept during the whole experiment in a metal cages under hygienic conditions and water adlibitum.

Rats were weighed and blood samples were obtained at time intervals 15, 30 and 45 days. Serum was separated then subjected to the serum biochemical analyses.

At the end of the experiment, rats were sacrificed and organs were excised and weighed.

Chemical analyses

Moisture, protein, ash, carbohydrate and crude fibre contents of the soup mixtures were determined according to **AOAC (1995).**

Serum biochemical analyses

Separated serum was used for the following biochemical analyses: total protein was determined according to the method of **Henry (1964),** alkaline phosphatase activity was determined according to the method of **Demetrious (1974),** total calcium was determined according to the method of **Robertson (1968),** ionized calcium was determined using Blood Gas Payar apparatus 564, and phosphorous was determined according to the method of **Fiske and Subbarow (1925).**

Statisical analysis

Data were analyzed by ANOVA and significant difference among means were separated by using LSD test according to **Newman and Federer (1963).**

RESULTS AND DISCUSSION

Two types of soup (lentil and vegetable) were prepared and used as calcium fortified products for people who needed to consume diets supplying the recommended calcium intake. **Table (1)** shows the proximate analysis of the prepared soups. Lentil soup contained more protein and carbohydrate and less fiber than vegetable soup.

To study the effect of calcium enriched diet, ovariectomized female albino rats were fed for 30 days on low calcium diet to produce experimental osteoprotic animals. Changes in the biochemical parameters of serum of the osteoporotic animals are shown in **Table (2).**

Table 1. Proximate analyses of lentil and vegetable soups

% of components	Lentil soup	Vegetable soup
Total protein	32.60	24.20
Total fat	1.29	1.29
Total corbohydrate	59.00	56.60
Ash	0.97	0.94
Moisture	2.29	2.84
Fiber	3.85	14.13

Table 2. Changes in some biochemical parameters in female albino rats fed on low-calcium-diet for 30 days. (n = 8 rats)

Parameters	Initial group	Osteoporotic group
Serum:		
Total protein (g/100ml)	6.59±0.08	5.66*±0.07
Alkalinephosphatase activity (μ/L)	81.23±1.44	141.02***±0.49
Total calcium (mg/100ml)	14.63±0.24	10.71**±0.18
Ionized calcium (mg/100ml)	5.12	3.44
Phosphorus (mg/100ml)	7.94±0.10	4.47***±0.13
Organs weight (g):		
Liver	4.76±0.16	3.95±0.06
Kidney	0.81±0.03	0.90±0.04
Heart	0.42±0.03	0.45±0.02
Lung	0.96±0.03	1.01±0.06
Spleen	0.55±0.03	0.40±0.04

Mean ± S.E * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Significant decrease in serum total protein ($P < 0.05$) and a highly ($P<0.01$) and very highly significant ($P < 0.001$) decreases in total calcium and phosphorus were observed respectively, in comparison with the initial group. A decrease in serum ionized calcium was also observed. Contraversely a very highly significant increase was observed in serum alkaline phosphatase activity of rats at the end of low calcium diet period in comparison with

the initial group. The decrease in free-ionized calcium concent-ration and the increase in alkaline phosphatase activity in serum may be attributed to the hyperparathyroidism. This hyperthyroidism resulted from calcium deficiency produced from feeding ovariectomized rats on low calcium diet for long period **(Zilva, *et al* 1994).**

The results in **Table (2)** showed also non significant changes in relative organs weight (liver, kidney, heart, lung and spleen) to body weight in comparison with the initial group at the end of low calcium feeding period. These results were in agreement with **Omi *et al* (1994) and Hamalainen (1994).**

The effect of feeding the osteoporotic rats with different calcium fortified formulas for 45 days on the body weight was illustrated in **Table (3)**. The data show a very highly significant ($P < 0.001$) increase in body weight during experimental diet period in comparing to the initial values. The increase was a function of the type of diet formula. From the data, it can be seen that diet formulas contained lentil soup (groups control^{++}, IV, V and VI), gave the highest body weight gain among all treated group (43-46%), which may be attributed to the high content of protein and carbohydrate in lentil soup.

Non significant changes were observed in relative organs weight to body weight in all groups at the end of experimental feeding period in comparison with the control group **(Table 4)**.

Osteoporotic rats, produced from feeding with low calcium diet for 30 days, showed a significant lower figure in serum protein in comparison with the initial group **(Table 2)**. The importance of serum protein in calcium metabalism was attributed to that, 45% of blood calcium circulates in the blood in form of calcium bound proteins **(Gurr, 1999)**.

Feeding the osteoporotic rats with different diet formulas and different calcium sources demonstrates changes in serum biochemical parameters during experimental period. **Tables (5, 6 and 7)** show the change in serum protein of osteoporotic rats during experimental feeding period and the outcome of the net results, produced from the statistical analysis.

The results showed that all treated groups recovered their serum protein after 30 days feeding period and exceeded the initial value at the end of feeding period, the highest increasing value was observed in serum protein – which is important for calcium metabolism – when oyster shell was used as calcium source. Calcium citrate come in the second place and egg shell come in the last place.

Osteoporotic rats produced from feeding with low calcium diet showed a very highly significant elevation in serum alkaline phosphatase activity **(Table 2). Tables (8, 9 and 10)** illustrate the serum alkaline phosphatase activity in osteoporotic rats after feeding with different diet formulas and different calcium sources during experimental period. The feeding led to recovery. The data showed that all groups recovered the alkaline phosphatase activity and reached their normal values at the end of feeding period. The net results of ANOVA test showed non significant decrease in all groups in comparison with the control group and also non significant changes was found between treated groups.

Osteoporotic rats showed a very highly significant depression in serum total colcium **(Table 2).** The effect of feeding the osteoporotic rats with different diet formulas and different calcium sources on serum total calcium was illustrated in **Tables (11, 12 and 13)**.

From the results we can observed that groups which were fed on formulas containiag vegetable soups showed slightly higher level in serum calcium content than the other groups. **Gurr, (1999)** mentioned that the proportion calcium absorbed from foods depends on how the calcium is chemically bound in the food and the presence of many substances present in the food, which may either enhance or inhibit absorption.

The data in **Tables (11, 12 and 13)** showed also that the osteoporotic rats recovered the serum calcium content at the end of feeding period in all groups. The level of serum calcium reached approximately its normal values. These results came in agreement with **Hamalainen, (1994)** who mentioned that low serum calcium found in calcium deficient rats was reversed by the administration of calcium salts.

The previous data is also in agreement with **Marie *et al* (1999)** who mentioned that, plasma calcium or blood calcium which accounts for only 0.1% of the body calcium, is kept at a remarkably stable level. When the blood calcium concentration is too low, parathyroid hormone and/or calcitriol bring blood calcium concentration up to the required level by mobilizing calcium form bone, increasing its absorption or encouraging its resorption from the kidneys. When the blood calcium concentration is too high, calcitonin ensures that calcium is shifted back into bone or excreted in urine **(Michaelsen, 1994).**

Table 3. Body weight changes in osteoporotic female albino rats fed on different calcium fortified fromulas for 45 days. (n = 8 rats)

Animal groups▲	Feeding duration (days)			
	0	15	30	45
Control'	112.58±1.88	116.73±0.76	132.58±2.32	147.21±2.54
Group I	100.75±0.49	105.63±1.30	118.36±2.16	136.00±1.36
II	102.08±0.39	104.60±1.80	115.14±1.76	137.80±1.36
III	103.40±0.91	106.43±1.56	120.08±1.73	138.78±1.19
Control''	101.26±1.59	112.24±2.75	129.95±1.18	142.56±0.88
Group IV	110.53±1.14	118.81±1.43	136.50±0.93	153.53±1.13
V	111.50±2.72	116.24±1.96	133.90±1.43	157.6±1.72
VI	110.19±0.76	115.6±2.15	131.64±2.04	155.58±2.08
Control '''	101.00±0.56	106.7±1.05	129.95±1.18	138.76±1.19
Group VII	100.21±2.13	106.9±1.05	125.05±1.73	139.2±1.32
VIII	113.70±1.18	121.35±1.81	136.84±1.46	154.80±0.85
IX	101.00±0.56	105.26±0.94	117.78±1.54	136.70±1.05

Mean ± S.E.

▲- Animal groups are indicated previously in materials and methods section.

Table 4. Percentage of organs weight/body weight of osteoporotic female aibino rats fed on different calcium fortified formulas for 45 days. (n = 8 rats).

Animal▲ groups'	Liver	Kidney	Heart	Lung	Spleen
Control'	3.66±0.05	0.75±0.01	0.55±0.01	1.12±0.01	0.54±0.03
Group I	3.63±0.09	0.78±0.01	0.52±0.01	1.34±0.03	0.52±0.01
II	4.00±0.12	0.77±0.02	0.48±0.01	1.31±0.02	0.60±0.01
III	3.79±0.09	0.69±0.01	0.48±0.01	1.31±0.02	0.60±0.01
Control''	3.75±0.06	0.72±0.01	0.44±0.01	1.21±0.02	0.51±0.01
Group IV	3.67±0.08	0.79±0.01	0.41±0.01	1.04±0.03	0.60±0.02
V	3.50±0.09	0.66±0.02	0.43±0.01	1.00±0.02	0.46±0.01
VI	3.40±0.08	0.74±0.01	0.50±0.02	1.11±0.01	0.51±0.01
Control '''	3.63±0.09	0.79±0.01	0.48±0.02	1.16±0.01	0.52±0.01
Group VII	3.79±0.06	0.77±0.01	0.46±0.01	1.18±0.04	0.54±0.01
VIII	3.58±0.14	0.70±0.02	0.46±0.01	1.16±0.01	0.56±0.02
IX	3.36±0.10	0.71±0.01	0.50±0.02	1.22±0.02	0.53±0.01

Mean ± S.E.

▲- Animal groups are indicated previously in materials and methods section

Table 5. Serum protein (g/100ml) in osteoporotic female albino rats fed on calcium fortified basal diet formulas for 45 days. (n = 8 rats).

Animal▲ groups	Initial group	Osteo-porotic group	Feeding duration (day)			Average during the feeding duration
		0	15	30	45	
Control'	6.59±0.18	5.66 ±0.17	5.85±0.17	5.93 ±0.16	6.24 ±0.21	5.92^{c}
Group I	6.59±0.18	5.66±0.17	6.54±0.18	7.02 ±0.24	7.53 ±0.32	6.69^{bc}
II	6.59±0.18	5.66±0.17	8.58±0.12	8.93 ±0.27	9.00 ±0.25	8.04^{a}
III	6.59±0.18	5.66±0.17	5.93±0.10	8.29 ±0.16	9.20 ±0.30	7.27^{ab}

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters.

Table 6. Serum protein (g/100ml) in osteoporotic female albino rats fed on basal diet and calcium fortified lentil soup formulas for 45 days. (n = rats)

Animal▲ groups	Initial group	Osteo-porotic group	Feeding duration (day)			Average during the feeding duration
		0	15	30	45	
Control''	6.59 ±0.18	5.66±0.17	5.85±0.16	7.45 ±0.11	7.80 ±0.21	6.69^{b}
Group IV	6.59 ±0.18	5.66±0.17	6.55±0.17	9.04 ±0.22	9.70 ±0.28	7.74^{ab}
V	6.59 ±0.18	5.66±0.17	8.84±0.29	9.87 ±0.22	9.75 ±0.30	8.53^{a}
VI	6.59 ±0.18	5.66±0.17	7.76±0.11	9.52 ±0.32	9.58 ±0.23	8.13^{a}

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters.

Table 7. Serum protein (g/100ml) in osteoporotic femal albino rats, fed on basal diet and calcium fortified vegetable soup formulas for 45 days. (n = 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Feeding duration (day)			Average during the feeding duration
		0	15	34	45	
Control'''	6.59±0.18	5.66±0.17	5.93±0.17	6.24±0.26	6.63±0.12	6.12^{c}
Group VII	6.59±0.18	5.66±0.17	6.24±0.12	7.14±0.26	7.80±0.20	6.71^{bc}
VIII	6.59±0.18	5.66±0.17	7.39±0.07	8.78±0.15	9.29±0.22	7.78^{a}
IX	6.59±0.18	5.66±0.17	6.32±0.14	7.33±0.20	8.96±0.21	7.06^{ab}

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters

Table 8. Serum alkaline phosphatase activity (μ/L) in female albino rats fed on calcium fortified basal diet formulas for 45 days. (n= 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Time intervals (day)			Average during the feeding duration
		0	15	30	45	
Control+	81.23±1.18	141.02±2.27	138.85±1.99	124.53±2.21	104.65±2.35	127.26 a
Group I	81.23±1.18	141.02±2.27	130.10±2.67	117.01±2.16	98.30±1.46	121.61 a
II	81.23±1.18	141.02±2.27	138.85±1.99	107.40±2.16	90.9±0.96	119.55 a
III	81.23±1.18	141.02±2.27	124.53±2.21	112.50±2.07	98.37±2.90	119.11 a

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters

Table 9. Serum alkaline phosphatase activity (μ/L) in osteoporotic female albino rats fed on basal diet and calcium fortified lentil soup formulas for 45 days. (n= 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Time intervals (day)			Average during the feeding duration
		0	15	30	45	
Control++	81.23±1.18	141.02±2.27	126.50±2.57	103.99±1.44	100.13±2.15	117.91 a
Group IV	81.23±1.18	141.02±2.27	123.85±1.99	101.63±1.56	79.39±0.61	111.47 a
V	81.23±1.18	141.02±2.27	124.53±2.21	109.63±2.56	81.63±2.67	114.20 a
VI	81.23±1.18	141.02±2.27	127.31±1.27	103.45±1.53	83.85±0.63	113.91 a

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters.

Table 10. Serum alkaline phosphatase activity (μL) in osteoporotic female albino rats fed on basal diet and calcium fortified vegetable soup formulas for 45 days. (n= 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Time intervals (day)			Average during the feeding duration
		0	15	30	45	
Control+++	81.23±1.18	141.02±2.27	135.31±1.99	126.10±2.67	103.99±1.44	126.61 a
Group VII	81.23±1.18	141.02±2.27	136.57±1.72	123.49±2.04	89.87±0.54	122.74 ab
VIII	81.23±1.18	141.02±2.27	131.02±2.27	116.96±2.99	86.86±1.51	118.97 ab
IX	81.23±1.18	141.02±2.27	112.10±1.66	101.90±1.06	95.49±0.84	112.63 b

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any to averages have the same letters

Table 11. Serum total calcium (mg/100ml) in osteoporotic female albino rats, fed on calcium fortified basel diet formulas for 45 days. (n = 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Feeding duration (day)			Average during the feeding duration
		0	15	30	45	
Control+	14.63±0.24	10.71±0.18	10.59±0.25	11.80±0.21	13.71±0.20	11.70[ab]
Group I	14.63±0.24	10.71±0.18	10.21±0.34	11.40±0.42	12.50±0.17	11.21[b]
II	14.63±0.24	10.71±0.18	10.39±0.17	11.40±0.33	14.00±0.23	11.63[ab]
III	14.63±0 24	10.71±0.18	10.91±0.29	13.95±0.24	15.08±0.31	12.66[a]

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters

Table 12. Serum total calcium (mg/100ml) in osteoporotic female albino rats, fed on basal diet and calcium fortified lentil soup formulas for 45 days. (n = 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Feeding duration (day)			Average during the feeding duration
		0	15	30	45	
Control++	14.63±0.27	10.71±0.18	10.59±0.25	12.83±0.21	14.77±0.20	12.23[a]
Group IV	14.63±0.27	10.71±0.18	10.93±0.23	14.85±0.28	14.80±0.31	12.82[a]
V	14.63± 0.27	10.71±0.18	10.29±0.27	12.65±0.23	14.55±0.39	12.05[a]
VI	14.63±0.27	10.71±0.18	10.36±0.26	13.30±0.33	14.66±0.31	12.26[a]

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters

Table 13. Serum total calcium (mg/100ml) in osteoporotic female albino rats, fed on basal diet and calcium fortified vegetable soup formulas for 45 days. (n = 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Feeding duration (day)			Average during the feeding duration
		0	15	30	45	
Control+++	14.63±0.27	10.71±0.18	10.70±0.30	11.20±0.28	14.52±0.25	11.72[b]
Group VII	14.63±0.27	10.71±0.18	10.99±0.24	12.98±0.20	14.82±0.21	12.38[ab]
VIII	14.63±0.27	10.71± 0.18	11.45±0.21	15.35±0.18	16.28±0.37	13.45[a]
IX	14.63±0.27	10.71 -0.18	11.80±0.27	14.55±0.25	15 73±0.55	13.19[a]

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters

Calcium circulates in the blood in three main forms: bound to proteins (about 45%), complexes with citrate, phosphate or bicarbonate (about 10%) and as free calcium ions (ca^{++}), (about 45%). The ionized form is physiologically important, and its concentration is regulated through the integrated actions of parathyroid hormone, calcitriol and calcitonin, the concentration of these hormones, respond to changes in concentration of calcium ions in the plasma by a process of "negative feedback", This process regulates the concentration of calcium in blood within narrow limits. (**Gurr, 1999**).

The osteoporotic rats, produced from feeding with low calcium diet for 30 days, showed a significant decrease in serum ionized calcium (**Table 2**). The data in **Table (14)** indicates the effect of feeding the osteoporotic rats with different diet formulas, fortified with different calcium sources for 45 days on serum ionized calcium. The data show that serum ionized calcium was not completely recoverd at the end of feeding period. Groups II, III, V, VI, VIII and IX which were fed on diet containing oyster shell or calcium citrate as calcium source, showed more recovery in serum ionized calcium than that in serum of groups I, IV and VII which were fed on formulas containing egg shell as calcium source.

Osteoporotic rats had a very highly significant lower figure in serum phosphorus (**Table 2**). **Tables (15, 16 and 17)** showed, the effect of feeding the osteoporotic rats with different diet formulas and different calcium sources on serum phosphorus during experimental feeding period.

Table 14. Serum ionized calcium (mg/100ml) in osteoporotic female albino rats, feeding with different diet formulas and different calcium sources for 45 days at the end of feeding period

Animal groups▲	Ionized calcium (mg/100ml)
Initial	5.12
Osteoporotic	3.44
Control $^{+}$	3.91
Group I	4.40
II	5.20
III	4.84
Control $^{++}$	3.50
Group IV	4.00
V	4.72
VI	4.44
Control $^{+++}$	3.62
Group VII	3.84
VIII	4.56
IX	4.24

▲- Animal groups are indicated previously in materials and methods section

Table 15. Serum phosphorus (mg/100ml) in osteoporotic female albino rats, fed on calcium fortified basal diet formulas for 45 days. (n = 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Feeding duration (day)			Average during the feeding duration
		0	15	30	45	
Control^{+}	7.94±0.10	4.47 ±0.13	4.94±0.11	6.47±0.11	8.89±0.21	6.19^{a}
Group I	7.94±0.10	4.47 ±0.13	4.29±0.12	6.55±0.14	9.52±0.19	6.21^{a}
II	7.94±0.10	4.47 ±0.13	4.39±0.01	7.84±0.14	9.84±0.15	6.63^{a}
III	7.94±0.10	4.47 ±013	4.11±0.12	7.54±0.23	8.63±0.15	6.19^{a}

LSD ($P > 0.05$)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference ($P > 0.05$) between any two averages have the same letters.

Table 16. Serum phosphorus (mg/100ml) in osteoporotic female albino rats, fed on basal diet and calcium fortified lentil soup formulas for 45 days. (n = 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Feeding duration (day)			Average during the feeding duration
		0	15	30	45	
Control++	7.94±0.10	4.47 ±0.13	4.29±0.12	6.45±0.14	8.00±0.21	5.80[b]
Group IV	7.94±0.10	4.47 ±0.13	4.32±0.07	6.65±0.10	7.79±0.11	5.81[b]
V	7.94±0.10	4.47 ±0.13	5.74±0.13	8.36±0.12	9.44±0.14	7.00[a]
VI	7.94±0.10	4.47±013	5.92±0.09	7.65±0.13	9.57±0.16	6.90[a]

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters.

Table 17. Serum phosphorus (mg/100ml) in osteoporotic female albino rats, fed on basal diet and calcium fortified vegetable soup formulas for 45 days. (n = 8 rats)

Animal▲ groups	Initial group	Osteo-porotic group	Feeding duration (day)			Average during the feeding duration
		0	15	30	45	
Control+++	7.94±0.10	4.47±0.13	4.32±0.07	6.32±0.12	8.13±0.16	5.81[b]
Group VII	7.94±0.10	4.47±0.13	4.26±0.12	6.00±0.12	8.21±0.16	5.74[b]
VIII	7.94±0.10	4.47±0.13	6.47±0.12	8.26±0.22	9.60±0.25	7.20[a]
IX	7.94±0.10	4.47±013	6.18±0.13	7.99±0.12	8.47±0.34	6.78[a]

LSD (P > 0.05)
Mean ± S.E.
▲- Animal groups are indicated previously in materials and methods section.
a,b,c there is no significant difference (P > 0.05) between any two averages have the same letters.

The data showed that all groups recoverd their serum phosphorus at the end of the experimental period, but the highest recoveries were observed in the groups, which received oyster shell as calcium source in the different diet formulas, especially in the presence of soups. Calcium citrate come in the second place and egg shell come in the last place.

The aforementioned results were in agreement with **Hamalainen, (1994)** who had reported that the calcium deficient rats excreted magnesium and phosphate in urine in high concentration and most of these changes were reversed by the administration of calcium salts.

From the study it can be concluded that egg shell is a cheap and good utilizable source for calcium.

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تقييم بعض الأغذية المدعمة بالكالسيوم للوقاية من هشاشة العظام

[٢٨]

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تم في هذا البحث التقييم البيولوجي بإستخدام أغذية مدعمة بمصادر مختلفة من الكالسيوم والمنتجة بهدف إستخدامها للوقاية من هشاشة العظام. لذلك تم إعداد شوربة عدس، شوربة خضار مدعمة بـ (قشر البيض - غلاف المحار - سترات الكالسيوم) كمصدر للكالسيوم واستخدامها في إعداد وجبات غذائية لفئران التجارب. وقد استخدمت فئران التجارب المصابة بهشاشة العظام الناتجة عن تغذيتها على غذاء منخفض في الكالسيوم لمدة ثلاثون يوما. ثم تم تغذيتها على الوجبات المدعمة بالكالسيوم لمدة ٤٥ يوم تالية، وتم أخذ عينات من مصل الدم في بداية التجربة وعلى فترات كل ١٥ يوم حتى ٤٥ يوم وذلك لإجراء التحاليل البيوكيميائية لتقدير كل من البروتين الكلي، والبروتين الذائب، نشاط انزيم الفوسفاتيز القاعدي، الكالسيوم الكلي، الكالسيوم المتأين، الفوسفور.

وقد أظهرت النتائج بالفحص إستعادة الفئران للنسب الطبيعية لمكونات سيرم الدم في نهاية مدة التغذية والتي سبق تغيرها معنويا بالفئران المصابة بهشاشة العظام وذلك عند مقارنتها بمجموعات الفئران المقارنة ، كما أظهرت التجارب تقارب النتائج الناتجة عن المصادر المختلفة للكالسيوم وإمكانية إستخدام قشر البيض كمصدر إقتصادي ومحلي للكالسيوم في الأغذية المدعمة والمستخدمة في الوقاية من هشاشة العظام.

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