

## A novel biogenic formulation for osteoporosis reduction and promotion of bone tissue formation

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### KEYWORDS

Eggshell powder, nano-CaCO<sub>3</sub>, Osteoporosis, Bone mineral density, Toxicity, biological evaluations, Histology study.

### SHORT SUMMARY

*Osteoporosis is one of the most significant serious bone diseases affecting human health. It is described by low bone mass as well as decreased bone mineral density that leads to bone fragility and fractures. This study aims to prepare and characterize the nano-CaCO<sub>3</sub> powder prepared from chicken eggshell (as a natural source for CaCO<sub>3</sub>). The studying and comparison of the effect of injectable solo nano-CaCO<sub>3</sub> as well as its combinations with vitamin D3 and omega-3 or commercial drug Alendronate (the usual drugs used for curing osteoporosis) on the curing of osteoporosis will be carried out. The results of the TEM examinations declared the presence of CaCO<sub>3</sub> in the nano form. It showed irregular, nonhomogeneous structured sub-spherical particles having various sizes ranging between 9.86–33.40 nm. While the chemical analysis carried out by the XRF route confirmed that the eggshell powder could be considered a valuable source of pure natural carbonate-based material, which contains some beneficial trace elements such as strontium (Sr), zinc (Zn), copper (Cu), and Zr. Such elements have a great influence on the bone-building process. The in vivo experiments were conducted using adult male rats divided into seven groups (six rats/group). All the measurements were taken immediately after the oral injection and one and two months post-injection. Acute toxicity, biochemical analysis of serum calcium and phosphorus, and kidney and liver functions were tested. The bone formation ability and healing process were followed up through bone mineral density (BMD) measurement and histological examinations. The results indicated that all used materials have no toxicity effect on liver or kidney functions. Using eggshell and eggshell combination with vitamin D3 and omega-3 significantly impacted bone formation, even better than that noticed for the typically used commercial drugs.*

### EXTENDED ABSTRACT

Annually, many people suffer from osteoporosis, which is considered a severe bone disease. Osteoporosis originates from decreased bone density, leading to high fracture risk. This study aims at the preparation and characterization of the nano-CaCO<sub>3</sub> powder prepared from chicken eggshell (as a natural source for CaCO<sub>3</sub>). The studying and comparison of the effect of injectable solo CaCO<sub>3</sub> as well as its combinations with vitamin D3 and omega-3 or commercial drug Alendronate (the usual drugs used for curing

osteoporosis) on the curing of osteoporosis will be carried out.

**Methods:** The collected eggshells were cleaned with distilled water, dried, and then ground to pass 90 $\mu$  sieved as described by Naga et al [1]. The egg shell powder was freshly suspended in sterile distilled water with few drops of Tween 80 to be ready for use in injectable form of nano-CaCO<sub>3</sub> for oral injection of rats. The in vivo experiments were conducted using adult male rats divided into 7 groups (6 rats/group): **G1:** Control group; **G2:** induced osteoporosis group; **G3:** treated by

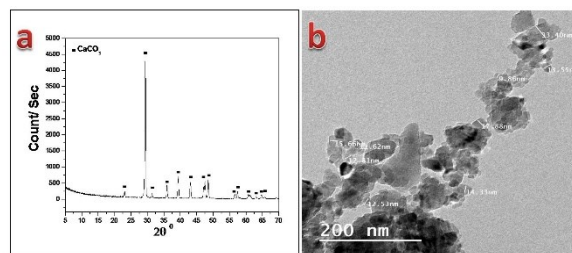
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commercial Alendronate 7 mg/kg drug; **G4**: treated with Eggshell 200 mg/kg as a drug; **G5**: treated with Omega3 200 mg/kg. **G6**: treated with Vit D 40 IU/kg and **G7**: combination group treated with eggshell 200 mg/kg + omega3 200 mg/kg + Vit D 40IU/kg. All the measurements were taken immediately after the oral injection and 1-2 months post-injection. Acute toxicity, biochemical analysis of serum calcium and phosphorus, and kidney and liver functions were tested. The bone formation ability and healing process were followed up through bone mineral density (BMD) measurement and histological examinations.

**Results:** The XRD pattern (**Fig 1a**) indicated the presence of calcium carbonate as a pure phase, while the TEM (**Fig 1b**) confirmed that CaCO<sub>3</sub> particle size is in the nano range powder with sizes ranging between 9.86–33.40 nm. The *in-vivo* results indicated that all used materials have no toxicity effect on liver or kidney functions as shown in (**Table 1**). Using eggshell and eggshell combination with vitamin D3 and omega-3 significantly impacted bone formation as shown in (**Figure 2d**), even better than that noticed for the typically used commercial drugs.

**Table 1** Biochemical analysis of the levels of liver and kidney functions, tumor markers, Ca and P ions concentrations measured in the serum of the different rat groups treated with the drugs under study.

Test	Time, Months	Groups treated by drugs						
		Normal (G1)	Osteoporosis (G2)	Alendronate 7 mg/kg (G3)	Eggshell 200 mg/kg (G4)	Omega 3 200 mg/kg (G5)	Vit D 40 IU/kg (G6)	Combination (G7)
CRE (mg/dl)	0	0.51±0.01	0.50±0.02	0.50±0.01	0.50±0.003	0.48±0.001	0.49±0.002	0.51±0.01
	1	0.54±0.02	0.48±0.09	0.56±0.02	0.50±0.001	0.47±0.021	0.50±0.025	0.47±0.05
	2	0.52±0.03	0.52±0.02	0.52±0.01	0.53±0.002	0.50±0.013	0.42±0.021	0.44±0.24
Urea (mg/dl)	0	40.31±2.65	39.77±3.80	37.99±2.78	38.44±4.25	40.44±3.66	36.44±3.21	35.77±2.95
	1	37.20±3.36	34.60±1.60	33.60±1.96	28.60±0.24	38.83±1.57	39.60±0.21	39.20±0.30
	2	40.50±3.39	37.17±4.41	36.20±1.56	38.20±3.25	40.50±2.07	40.20±1.42	40.33±3.04
ALT (U/ml)	0	56.87±2.98	44.85±4.98	59.44±4.87	62.47±3.70	47.12±2.55	50.44±4.98	40.78±7.14
	1	58.00±3.08	93.80±10.84	61.60±4.65	56.60±6.55	51.80±2.57	53.80±2.23	46.60±3.48
	2	68.60±5.71	106.0±10.42	93.60±6.54	69.40±5.71	92.40±2.611	82.80±2.15	72.00±2.99
AST (U/ml)	0	149.54±10.4	160.47±9.45	155.45±3.65	132.24±3.14	159.61±13.7	157.77±12.14	149.74±10.88
	1	154.2±5.36	241.60±30.14	244.00±24.88	160.80±7.87	178.30±9.55	181.20±5.75	160.60±9.29
	2	179.3±19.87	271.30±16.76	190.80±12.12	168.40±15.75	195.80±18.74	138.60±7.33	130.00±10.26
ALK.P (U/l)	0	288.41±23	324.15±32	310.41±21	249.44±11	266.77±18	360.70±23	247.14±13
	1	377.7±23	353.00±28	261.20±24	255.20±22	311.00±27	355.40±37	212.20±19
	2	393.3 ±23	337.30±22	281.21±25	262.43±22	287.01±26	359.81±16	255.80±21
Ca <sup>2+</sup> (mg/dl)	0	9.97±0.14	9.84±0.09	9.99±0.04	10.01±0.04	9.88±0.03	10.07±0.001	9.87±0.07
	1	9.92±0.142	8.29±0.17	9.52±0.21	9.16±0.18	9.20±0.07	9.08±0.02	9.23±0.06
	2	11.10±0.15	7.48±0.21	10.28±0.07	10.11±0.29	10.13±0.28	9.90±0.13	10.34±0.46
p <sup>4+</sup> (mg/dl)	0	7.12±0.12	6.98±0.54	7.84±0.38	6.88±0.48	7.55±0.32	7.14±0.38	7.24±0.87
	1	7.75±0.462	6.22±0.33	7.46±0.83	7.06±0.35	7.18±0.29	7.12±0.56	7.38±0.89
	2	6.18±1.06	3.70±0.17	6.52±0.63	7.18±0.65	6.25±0.38	5.02±0.55	7.89±0.32



**Figure 1** XRD pattern (a) and TEM image of eggshell powder (b).

**Conclusions:**

we recommended the use of eggshell powder alone or in combinations with vitamin D3 and omega-3 due to great potential for the treatment of bone osteoporosis.



**Figure 2** Graphical Abstract Experimental steps.

**References**

[1] S.M. Naga, H.F. El-Maghraby, M. Sayed, E.A. Saad, 2015, "Highly porous scaffolds made of nanosized hydroxyapatite powder synthesized from eggshells", J. Ceram. Sci. Technol 6 [3]: pp. 237-244.