



## **Effect of a mixture of shrimp peel extract and shellfish of some types of oysters on weight gain of experimental rats with osteoporosis induced with dexamethasone**

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**Abstract: Aim of the study:** This research aims to study the Effect of a mixture of shrimp peel extract and shellfish of some types of oysters on weight gain of experimental rats with osteoporosis induced with dexamethasone as a nutritional supplement to maximize the role of the Blue Economy in promoting sustainable development.

**Methods Used:** Shrimp peels extract (it is extracted by water and alcohol)

Oyster extract (extracted by water and alcohol)

**Measures, Biological Assessment:** A biological experiment is being carried out on experimental rats (albino rats) to evaluate the effect of feeding a mixture of shrimp shell extract and shellfish of some types of oysters on experimental rats with osteoporosis induced with dexamethasone. A number (50) of (albino rats) are used, distributed into five groups under test. Each group consists of ten (10) rats; (5) males and (5) females, All rats were fed with the standard food for a week before the start of the experiment to get adapted. Both the positive control and experimental groups of rats have to be injected intramuscularly with DEX (7 mg / kg of body weight) once a week for 4 weeks.

1- The first group, the healthy (negative) control group, feed on the basic food

2- The second group, the infected (positive) control group feed on the basic food

3- The third experimental group (1) feed on the basic food + extract of 500 mg of oyster shells + extract of 500 mg of shrimp shells.

4- The fourth group; the experimental group (2),are fed on the basic food + extract of 1600 mg of oyster shells + extract of 1600 mg of shrimp shells

5- The fifth group; the experimental group (3),are fed on the basic food + extract of 3000 mg of oyster shells + extract of 3000 mg of shrimp shells.

**Method Used:** Weight gain using medical electronic scale.

**Statistical Analysis:** The data are treated according to the SPSS program and the necessary statistical treatments,  $P=0.05$ .

**Conclusion:** The results of this study about the effects of a mixture of shrimp peels extract and shellfish of some types of oysters on experimental rats with osteoporosis induced with dexamethasone revealed the following conclusions:

1. There are different results for different management of groups
2. The male rats were higher in weight gain compared to female rats

**Recommendation:** It is recommended that Bio-economic importance of shrimp and oyster shell due to the benefit of chitosan and chitin leading to weight gain and benefit from the waste in agriculture and biotechnology industry, wastewater treatment, Energy conversion and biomedical material development

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**Key words:** Shrimp peel extract and oysters; male and female rats; osteoporosis; dexamethasone.

### **Introduction**

Osteoporosis represents a major health burden affecting millions of people all over the world, and is one of the most common metabolic disorders associated with disabilities and deaths as it is a chronic and progressive bone disease associated with a decrease in bone mass and a subtle weakening of the

bone tissue, which leads to osteoporosis and increased risk of fractures.

Osteoporosis is classified as one of the common diseases of aging after diabetes, high blood pressure and heart disease according to the World Health Organization.

Osteoporosis can begin at an early age of 25 years. However, the majority of people with

osteoporosis are postmenopausal women, so it is important to promote educational interventions designed to prevent osteoporosis by providing health education about lifestyle modification such as regular exercise, exposure to sunlight, maintaining a normal body weight, eating foods rich in calcium and vitamin D to prevent osteoporosis. Taking these preventive measures in a timely manner reduces the incidence of osteoporosis and prevents fractures.

Shrimp peel is a food source rich in proteins, vitamins, mineral salts and other compounds that stimulate blood circulation in the human body. It also contains many antioxidants such as beta-carotene and others that prevent and eliminate free radicals. Eating this extract regularly and in large quantities works to preserve the skin and hair, the freshness and nourishment of the skin, and prevents the appearance of wrinkles and blackheads. (Al-Ali, 2021)

Shrimp peels contain useful chemical nutrients such as calcium, phosphorous, protein and vitamin D. It is also an important source of vitamin B12, which is important for red blood cells and nerves, and its ratio is 30 micrograms of vitamin B12 per 100 grams of wet weight. We purified vitamin B12 compounds from extracts of shrimp muscles and head parts (Okamoto et al., 2020)

Shrimp is also a source of omega-3 and minerals such as zinc that are important for maintaining cell health, division and renewal.

The table shows some of the nutritional value contained in 100 grams of shrimps, which provides the benefits of shrimps as follows:

Nutrients	Nutritional value
Calories	85 calories
Protein	20 g
Fat	0.5 g
Cholesterol	161 mg
Calcium	64 mg
Magnesium	35 mg
Sodium	566 mg
Phosphorous	212 mg
zinc	1.3 mg

(El-Malky, 2017)

Nutrients	Nutritional value
Calories	102 calories
water	77.3 milliliters
Fat	3.4 mg
Calcium	118 mg
Iron	8.26 mg
Magnesium	36 mg
Phosphorous	145 mg
Potassium	280 mg

Sodium	510 mg
Zinc	78.26 mg
Vitamin A	23 micrograms
Vitamin B12	15.68 mg
Vitamin K	2. micrograms
Cholesterol	80 micrograms
Carbohydrates	5.42 mg

(Yoshikazu, 2018)

Rats received intramuscular injection of DEX (7 mg/kg b.wt.) once a week for 4 weeks, whereas DPE (150 mg/kg b.wt.) was given orally for the same duration. Results: DEX-treated rats exhibited significant decline in the body weight accompanied by marked reduction in serum and bone minerals (Ca, P), bone mineral density (BMD) and serum total protein (TP) with elevation in serum creatinine (CR) level (Hassan, 2019).

#### Definition:

In rats, weight gain refers to the increase in total body mass over time. It is typically measured by regularly weighing the animal and comparing to earlier weights. Weight gain includes increases in fat mass (adiposity), lean mass (muscle, organs), water, possibly bone, etc. (WHO, 2006)

In experimental settings, weight gain is often employed as:

An outcome measure (e.g., to see effect of diet, drugs, environmental exposure).

Part of defining or modeling obesity or growth.

A component of calculating feed efficiency, obesity indices (like Lee's Index), and body composition.

#### How it is measured in studies:

Weighting schedule: Rats are weighed at specific intervals (e.g., daily, weekly). Some studies monitor weight gain in grams/day.

Body composition: To distinguish what portion of weight gain is fat vs lean tissue. Methods include dual-energy X-ray absorptiometry (DXA), dissection of fat depots, etc.

Derived indices: For obesity models, e.g. fat/body weight ratio; Lee's Index (which relates weight to body length) is used (Hall and Hall, 2021).

**Key factors influencing weight gain in rats: Several factors affect how much and how quickly rats gain weight in experiments (CDCP. 2000):**

1. Diet composition

High-fat vs high-carbohydrate diets: more dietary fat tends to lead to greater fat accumulation and faster weight gain. Caloric density of diet.

2. Amount of food intake (overfeeding) vs energy expenditure. If intake exceeds expenditure, weight gain occurs.

3. Strain and age of rat: different rat strains and ages gain weight differently. Young rats (post-weaning) tend to grow rapidly. Older ones less so. Some are more prone to obesity.

4. Environment: Temperature: cooler environmental temperature can increase weight gain/fat accumulation in some contexts.

Stress: influences energy balance and may affect weight gain.

5. Activity/exercise: More physical activity tends to reduce weight gain or slow its rate.

6. Genetic or developmental programming: Maternal diet, early life nutrition, exposure to toxins, etc.

#### **Example values:**

Here are some values reported in studies (NIH, 2021): In one high-fat/high-sucrose feeding study, male rats exposed from weaning reached ~10% higher body weight than controls by ~20 weeks.

In the obesity model induced by improved high-calorie diet, rats had significantly higher weight gain compared to controls over 6 weeks; also showed increases in body fat, fat/body weight ratio.

In another study, when rats are housed individually vs grouped, weight gains of ~2.7-3.6 g/day have been observed under certain conditions.

#### **Aim of the Study**

This research aims to study the Effect of a mixture of shrimp peel extract and shellfish of some types of oysters on weight gain of experimental rats with osteoporosis induced with dexamethasone as a nutritional supplement to maximize the role of the Blue Economy in promoting sustainable development.

#### **Material and Methods**

##### **Materials:**

- Shrimp peels

- Shells of some types of oysters available in the study environment, such as clams and mussels.

That is by using food waste from each of (special food industry factories, civil society consumption, including (restaurants, markets, household consumption)

- Experimental rats from (Helwan Farm, Ministry of Health, Cairo, Egypt)

- Chemicals: They will be obtained from the General Company for Trade and Chemicals

##### **Methods Used:**

Shrimp peels extract (it is extracted by water and alcohol)

Oyster extract (extracted by water and alcohol)

##### **Measures:**

##### **Biological assessment:**

A biological experiment is being carried out on experimental rats (albino rats) to evaluate the effect of feeding a mixture of shrimp shell extract and shellfish of some types of oysters on patients with osteoporosis by experimental rats induced with dexamethasone. A number (50) of (albino rats) are used, distributed into five groups under test. Each group consists of ten (10) rats; (5) males and (5) females, All rats were fed with the standard food for a week before the start of the experiment to get adapted. Both the positive control and experimental groups of rats have to be injected intramuscularly with DEX (7 mg / kg of body weight) once a week for 4 weeks. (Hassan, 2019)

1- The first group, the healthy (negative) control group, feed on the basic food

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3- The third experimental group (1) feed on the basic food + extract of 500 mg of oyster shells + extract of 500 mg of shrimp shells.

4- The fourth group; the experimental group (2), are fed on the basic food + extract of 1600 mg of oyster shells + extract of 1600 mg of shrimp shells

5- The fifth group; the experimental group (3), are fed on the basic food + extract of 3000 mg of oyster shells + extract of 3000 mg of shrimp shells.

##### **Weight gain**

Was estimated by medical electronic scale

##### **Extraction of Shrimp Shells:**

Shrimp shells and heads weighing 3 kg were dried using hot air in an oven at a temperature of 40 degrees Celsius for 5 hours. After drying, the shells and heads were finely ground using a mill with a power of 3000 watts. The dried powder was then extracted using a mixture of water and methanol, with 50% water and 50% methanol at room temperature, and the extraction continued until completion to ensure maximum extraction of the extractable components. The methanolic extracts were then concentrated under reduced pressure using a rotary evaporator to remove the solvent. This concentration resulted in a viscous brownish-white crude extract weighing a total of 213 grams. The process was performed on 21 kg of shrimp shells and heads, yielding a total extraction weight of 1690 grams.

##### **Extraction of Oyster Shells:**

Oyster shells weighing 2 kg were dried using hot air in an oven at a temperature of 60 degrees Celsius for 4 to 5 hours. After drying, the oyster shells were finely ground using a mill with a power of 3000 watts. The dried powder was then extracted using a mixture of water and methanol, with 50% water and 50% methanol at room temperature, and the extraction continued until completion to ensure maximum extraction of the extractable components. The methanolic extracts were then concentrated under

reduced pressure using a rotary evaporator to remove the solvent. This concentration resulted in a viscous brownish-white crude extract weighing a total of 90 grams. The process was performed on 40 kg of oyster shells, yielding a total extraction weight of 1750 grams (<https://www.mdpi.com/1420-3049/27/19/6304>).

#### The Experiment:

A total of 25 male and 25 female rats were brought in and divided into 10 groups, with each group consisting of 5 rats.

#### Male Groups:

- Group One (Control Group): Consists of 5 male rats fed only basic food and water.
- Group Two (Negative Control Group): Consists of 5 male rats suffering from osteoporosis, fed only basic food and water.
- Group Three: Consists of 5 male rats suffering from osteoporosis, fed basic food with the addition of a mixture containing 500 mg of shrimp shell extract and 500 mg of oyster extract.
- Group Four: Consists of 5 male rats suffering from osteoporosis, fed basic food with the addition of a mixture containing 1600 mg of shrimp shell extract and 1600 mg of oyster extract.
- Group Five: Consists of 5 male rats suffering from osteoporosis, fed basic food with the addition of a mixture containing 3000 mg of shrimp shell extract and 3000 mg of oyster extract.

#### Female Groups:

- Group One (Control Group): Consists of 5 female rats fed only basic food and water.
- Group Two (Negative Control Group): Consists of 5 female rats suffering from osteoporosis, fed only basic food and water.
- Group Three: Consists of 5 female rats suffering from osteoporosis, fed basic food with the addition of a mixture containing 500 mg of shrimp shell extract and 500 mg of oyster extract.
- Group Four: Consists of 5 female rats suffering from osteoporosis, fed basic food with the addition of a mixture containing 1600 mg of shrimp shell extract and 1600 mg of oyster extract.
- Group Five: Consists of 5 female rats suffering from osteoporosis, fed basic food with the addition of a mixture containing 3000 mg of shrimp shell extract and 3000 mg of oyster extract (<https://www.ncbi.nlm.nih.gov/books/NBK231925/>)

Basic Diet for Rats:

Rodent diet number 3

Composition percentages:

- Casein: 4.12%
- Corn: 70.24%
- Soy: 8.80%
- Bran: 14.34%
- Vitamins: 1.00%
- Minerals: 1.00%

- Salt: 0.50%

Inducing Osteoporosis in Rats: The process involves inducing osteoporosis in rats by injecting them with 7 mg of dexamethasone solution weekly for two months to induce the condition.

Weight of the rats 1-6 weeks 7-12 weeks

Measuring of T.cal, L.cal vitamin D3.

Histopathology of liver and kidney.

Histopathology of the knee joint of male and female rats.

X-ray of the left hand of rats.

#### Determination of total protein:

Crude protein ( $N \times 6.25$ ) using the Kjeldahl method and total ash of samples were determined (AOAC, 2007).

#### Determine minerals

The contents of minerals such as: sodium was determined by the flame photometry method (Jahan *et al.*, 2011). Calcium and phosphorous were determined by flame atomic absorption spectrometry (Kirk and Sawyer, 1991).

#### HPLC conditions for Determine vitamins D3 and D2

Vitamins D3 (Cholecalciferol) and Vitamin D2 (Ergocalciferol) were determined in samples (shrimp peel and Oysters Shell) by performing HPLC analysis using an Agilent 1260 series (Frag *et al.*, 2018); separation was performed using Kromasil 100-5-C18 (4.6 mm x 250 mm ID, 5  $\mu$ m). The mobile phase consisted of water with 0.01% TFA (pH 2.9) (A) and Methanol (B) at a flow rate 1 ml/min. The mobile phase was programmed in case of fat-soluble vitamins consecutively in a linear gradient with standard vitamins D2 (Ergocalciferol) and D3 (Cholecalciferol) and the injection volume was 10  $\mu$ l. The multi-wavelength detector was monitored at 280 nm.

#### Determination of chitosan content for shrimp peel:

The chitosan content of shrimp shells was estimated by conducting two tests:

#### 1- Fourier Transform Infrared (FTIR) Spectroscopy:

The sample of shrimp peel to detect the presence of chitosan was characterized in KBr pellets by using an infrared spectrophotometer model (4100 Jasco, Japan) in the range of 400-4000  $\text{cm}^{-1}$  (Struszczyk, 1987).

#### 2- X-Ray powder diffraction (X-RD):

In order to confirm the crystalline or amorphous nature of chitosan for shrimp peel sample, X-RD pattern of shrimp peel sample was determined by X-Ray diffraction made by x, pert pro model PAN alytical Company, Netherland and data collection was performed using Cu anode and the voltage of the monochromator was 40 kV according to the methods of Pooja *et al.* (2014). The diffraction pattern was determined in the area  $2\theta > 2$ , the distinctive chitosan peaks are observed at  $^{\circ} 2$  theta.

### Elements contents of shrimp peel sample

The elements contents were evaluated for shrimp peel by using Energy Dispersive analysis of X-Ray Spectroscopy (JED-2300 analysis station, Joel). EDAX (Energy Dispersive analysis of X-Ray Spectroscopy) analysis used for chitosan characterization by (JED-2300 analysis station, Joel).

### Determination of chitosan content for shrimp peel: 1- Fourier Transform Infrared (FTIR) for detection chitosan in shrimp peel sample:

The FTIR peaks for shrimp peel sample was observed at  $3263.213093.76\text{ cm}^{-1}$  (N-H and O-H stretching vibrations),  $2919.35\text{-}2851.05\text{ cm}^{-1}$  (axial stretching of C-H in the polymer chain),  $1627.21\text{-}1558.30\text{ cm}^{-1}$  (amide I vibration modes),  $1480.09\text{-}1328.63\text{ cm}^{-1}$  (N-H straining vibrations of NH<sub>2</sub> groups),  $1203.91\text{ cm}^{-1}$  (CH<sub>2</sub> deformation vibrations),  $1154.24\text{ cm}^{-1}$  (symmetrical angular deformation of CH<sub>3</sub> in NHC(O)CH<sub>3</sub> groups),  $1067.89\text{-}1025.32\text{ cm}^{-1}$  (amide III vibration modes),  $952.70\text{ cm}^{-1}$  (C-O-C bridge),  $654.45\text{-}418.20\text{ cm}^{-1}$  (C-O stretching vibration of alcohol groups), respectively.

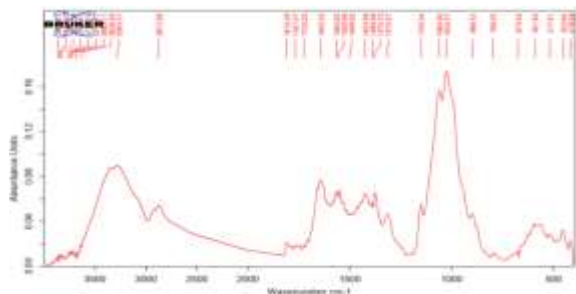


Fig. (1): FTIR peaks of chitosan content in shrimp peel sample

### 2- X- Ray powder diffraction (X-RD):

Fig. (2) Showed the characterization of chitosan from shrimp peel sample by X-ray diffraction (XRD) technique. The XRD studies of chitosan showed a sharper peak at  $40^\circ$  (1187.91 counts/s) and a maximum peak at  $30^\circ$  (2308.63 counts/s). Shrimp chitosan XRD pattern showed the sharpest peak at  $17.948^\circ$  (1267.05 counts/s) and the highest peak at  $10.745^\circ$  (2237.91 counts/s). The XRD studies of crab chitosan showed a sharper peak at  $25.421^\circ$  (4756.36 counts/s) and a maximum peak at  $14.835^\circ$  (8384.45 counts/s). The XRD results of the chitosan samples showed the crystalline nature of obtained polymers.

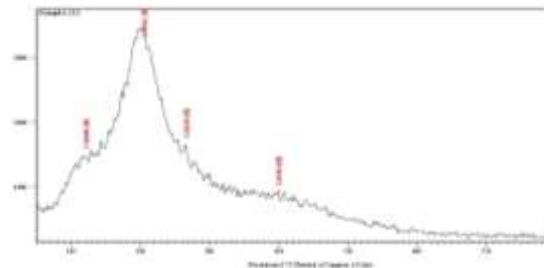


Fig. (2): The X-ray diffraction patterns (XRD) of chitosan content in shrimp peel sample

### Results FTIR and X-RD

#### Determination of Vitamin D2 and D3: The content of vitamin D3 (Cholecalciferol)

Sample	Vitamin D3		
	Reference Method	Units	Results
Shrimp peel	HPLC	$\mu\text{g}/100\text{ g}$	47.0338
Oysters Shell			39.2324

#### Standard curve

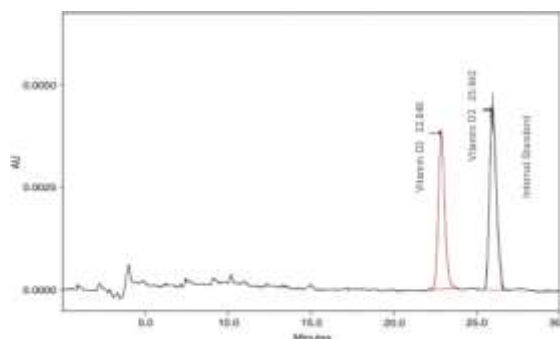
	Retention times (min)	Area	Conc. ( $\mu\text{g}/\text{ml}$ )
Vitamin D3	22.864	1384.62	100
Vitamin D2	25.942	1231.88	100

#### Shrimp peel sample

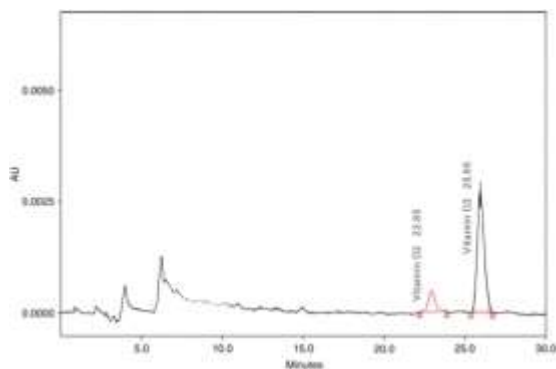
	Retention times (min)	Area	Conc. ( $\mu\text{g}/\text{g}$ )
Vitamin D3	25.95	651.24	47.0338
Vitamin D2	22.85	81.46	6.6126

#### Oysters Shell sample

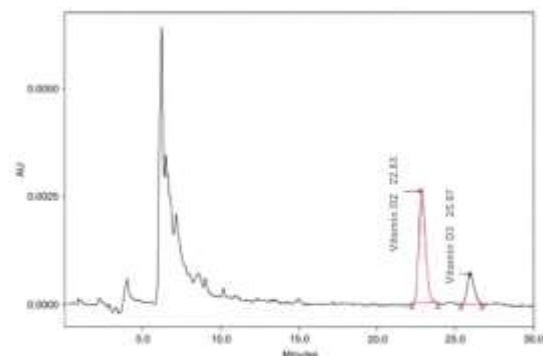
	Retention times (min)	Area	Conc. ( $\mu\text{g}/\text{g}$ )
Vitamin D3	25.97	543.22	39.2324
Vitamin D2	22.83	79.68	6.4682



HPLC of standard curve of vit D3 and D2



HPLC of vit D3 and D2 in shrimp peel



HPLC of vit D3 and D2 in Oysters Shell

**Statistical Analysis**

The data are treated according to the SPSS program and the necessary statistical treatments, P=0.05.

**Results**

Table (1): Male control weight

Time	M±SE
W1	109.8 ± 6.57
W2	115.8 ± 6.90 *
W3	124.6 ± 5.35 **
W4	126.4 ± 6.80 **
W5	127.4 ± 5.72 **
W6	130.0 ± 5.04 **

M ± SE, \* P = 0.05 significant  
\*\* P = 0.001 High, NS Non significant

Table (2): Weight of Rats:

1-6 Tables before Mixture ext of Shrimp and oysters shells Time Male Dexa 1

Time	Weight m±SE
1st week	122.8 ± 6.24
2nd week	121 ± 5.17
3rd week	126 ± 5.4 *
4th week	122.8 ± 5.43
5th week	129.2 ± 7.69 *

6th week	130.4 ± 8.99 *
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\* P = 0.05 significant, \*\* P = 0.001 High significant, NS Non significant

Table (3): Male Dexa weight

Time	M±SE
w1	118.2 ± 5.05
w2	119.2 ± 6.10
w3	123.5 ± 6.27
w4	122.6 ± 9.8
w5	129.8 ± 9.65 *
w6	134 ± 9.61 *

\* P = 0.05 significant, \*\* P = 0.001 High significant  
NS Non significant

Table (4): Weight Male Dexa 3

Time	M±SE
W1	134.8 ± 6.17
W2	131.2 ± 5.89 NS
W3	129.4 ± 6.74 NS
W4	125.6 ± 7.55 *
W5	123.0 ± 8.21 *
W6	122.6 ± 9.12 *

\* Significant, \*\* High significant  
NS: Non significant

Table (5): Male Dexa weight

Time	M+SE
w1	84.8 ± 2.46
w2	86.2 ± 2.14 NS
w3	90.4 ± 3.77 *
w4	90.6 ± 3.29 *
w5	97.2 ± 3.57 **
w6	100 ± 4.20 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (6): Female Control Weight

Time	M±SE
W1	80.2 ± 1.11
W2	87 ± 1.7 *
W3	97.7 ± 4.35 *
W4	104.8 ± 2.44 *
W5	110.4 ± 3.17 **
W6	120 ± 3.47 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (7): Weight of Females Rats 7-12 before the Mixture shrimp + oysters shells

Time	M±SE
W1	109 ± 2.66
W2	104 ± 4.06 *

W3	101.6 ± 3.52 *
W4	97 ± 5.07 *
W5	100.8 ± 6.31 *
W6	96.4 ± 9.07 *

\* Significant, \*\* High Significant, NS Non-Significant.

Table (8): Female Dexa 2

Time	M±SE
W1	88.4 ± 1.03 *
W2	86.2 ± 2.52 NS
W3	88.6 ± 1.60 NS
W4	84.8 ± 1.46 NS
W5	85.2 ± 3.43 NS
W6	87.0 ± 6.31 NS

\* Significant, \*\* High Significant, NS Non-Significant.

Table (9): Female Dexa (3)

Time	M±se
W1	104. ± 5.87
W2	101.2 ± 4.89
W3	103.4 ± 5.82
W4	100 ± 9.29
W5	109 ± 9.70 *
W6	113.4 ± 11.08 *

\* Significant, \*\* High Significant, NS Non-Significant.

Table (10) : Female Dexa (4)

Time	M±SE
W1	126.8±4.6
W2	125.2±7.25
W3	130.8±5.6 *
W4	133.4±4.20 *
W5	139.2±4.35 *
W6	146.8±6.15 *

\* Significant, \*\* High Significant, NS Non-Significant.

Table (11): Male Control (C-1)

Time	M±SE
W7	156 ± 11.07
W8	182.5 ± 19.77
W9	192.2 ± 21.17
W10	207.2 ± 24.2
W11	214.7 ± 23.11
W12	225 ± 23.14

\* Significant, \*\* High Significant, NS Non-Significant.

Table (12): Male Control (Dexa 4)

Time	M±SE
W7	123.6±2.33
W8	151±8.50 **
W9	160.6±4.66 **

W10	186±12.48 **
W11	222±27.61 **
W12	230.6±29.92 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (13): Weight of Rats during injection of the mixtures (shrimp, oysters) Male Dexa.1 500,500

Time	M ± SE
W7	151.2 ± 9.4
W8	178.7 ± 25.3 *
W9	191 ± 30.2 **
W10	192 ± 30.6 **
W11	199.7 ± 30.9 **
W12	205.7 ± 30.4 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (14) :Male Dex 2 1600/1600

Time	MISE
W7	154 ± 13.8
W8	173.3 ± 14.1 *
W9	184.3 ± 13.6 *
W10	194.1 ± 14.5 **
W11	204.6 ± 15.1 **
W12	222.3 ± 14.4 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (15): Male Dexa 3

Time	M±SE
W7	132.7±12.58
W8	147.5±13.41 *
W9	153.3±13.61 *
W10	169±13.02 *
W11	176.5±13.84 **
W12	195.7±13.91 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (16): Female Control (c\_1) weight 7-12 week

Time	M ± SE
W7	141.15 ± 6.23
W8	157.5 ± 4.79 *
W9	168.0 ± 6.26 *
W10	178.25 ± 5.66 *
W11	188.5 ± 6.59 **
W12	203.75 ± 10.29 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (17): Female Dexa 4 control

Time	M±SF
W7	162 ± 8.36
W8	177 ± 9.54 *

W9	189.3 ± 10.17 **
W10	198 ± 10.41 **
W11	205.67 ± 8.68 **
W12	214.3 ± 6.96 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (18): Female Dexa 1

Time	M±SE
W7	117 ± 11.35
W8	130 ± 8.72 *
W9	145 ± 2.89 **
W10	150.67 ± 4.41 **
W11	154.3 ± 3.84 **
W12	164.67 ± 4.19 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (19):Female Dexa 2

Time	M±SE
W7	120.3 ± 2.03
W8	145.3 ± 3.12 *
W9	154.0 ± 7.54 *
W10	166.6 ± 11.90 *
W11	173.3 ± 13.96 **
W12	178.1 ± 8.62 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (20): Female Dexa 3

Time	M+S.E.
W7	120 ± 27.01
W8	137 ± 27.01 *
W9	164 ± 28.10 **
W10	177.5 ± 32.50 **
W11	181.5 ± 31.65 **
W12	186.5 ± 33.49 **

\* Significant, \*\* High Significant, NS Non-Significant.

Table (21): Indicating total protein of the mixture

Sample	Total Protein g/100 g		
	Reference Method	Units	Results
Shrimp peel	Kjeldahl Method	g/100 g	25.81
Oysters Shell			5.17

#### Determination of total protein:

Crude protein ( $N \times 6.25$ ) using the Kjeldahl method and total ash of samples were determined (AOAC, 2007).

Table (22): Indicating the content of minerals per 100 g

Macro-elements	Reference Method	Units	Shrimp peel	Oysters Shell
Calcium (Ca)	Atomic Absorption	mg/100 g	89.21	46.830
Phosphorus (P)	Atomic Absorption	mg/100 g	34.90	1.961
Sodium (Na)	Atomic Absorption	mg/100 g	935.18	178.952

#### Determine minerals

The contents of minerals such as: sodium was determined by the flame photometry method (Jahan *et al.*, 2011). Calcium and phosphorous were determined by flame atomic absorption spectrometry (Kirk and Sawyer, 1991).

#### Results

Table (1) represents (M + SE) of tables before injections of the mixture extracts (shrimp and oysters shell) and (6) tables thereafter the injections of the mixture extracts.

Tables (1-6) mixture extracts represents the weight of Rats Males, Females.

In case of the weight of male rats; in Male Control the weight begin by week (1) ( $109 \pm 6.57$ ) to gradual increased to week (6). in Dexa (1) dexamethasone administration led to a drop of weight ( $84.8 \pm 2.46$ ) to reach ( $100 \pm 4.2$  g) after 6 weeks.

Male Dex (1) demonstrates an elevation of weight from ( $122.8 \pm 6.24$  g) week (1) to ( $130.4 \pm 8.99$  g) week (6).

Male Dex (2) the weight of week (1) began at ( $118.2 \pm 5.05$  g) to ( $134 \pm 9.61$  g) in week (6).

Male Dex (3) the weight began ( $131.8 \pm 6.17$  g) to week (6) ( $122.6 \pm 9.12$ ).

In case of the weight of rats before mixture, extract administration.

Female Control weight in week (1) ( $80.1 \pm 1.11$  g) to ( $120 \pm 3.47$  g) in case of week (6).

Female Dex (1) after dexamethasone administration ( $126.8 \pm 4.6$  g) week (1) reach ( $114.68 \pm 6.15$  g) week (6).

Female Dex (2) in week (1) the weight ( $109 \pm 2.66$  g) to reach ( $96.4 \pm 9.07$  g) week (6).

Female Dex (2) in week (1) weight ( $88.4 \pm 1.03$  g) to ( $87 \pm 6.31$ ) in week (6).

Female Dex (3) weight of week (1) ( $104.1 \pm 5.87$  g) to ( $113.4 \pm 11.08$  g) weight of week (6).

In case of weight of:

Female Control rats during injection of mixture extract in Week (1) [ $141.5 \pm 6.23$  g] to Week (12) [ $203.75 \pm 10.29$  g].

In case of Dex (1) dexamethasone administration (W7) [ $162. \pm 8.36$  gr] to (W12) ( $214.3 \pm 6.96$  gr)

In Dex (1) (500 mg + 500 mg) mixture

(W7) [ $117 \pm 11.35$  gr] to W12 [ $164.67 \pm 4.19$  gr]

In Dex (2) (1500 + 1500 mg) mixture

(W7) [120.3 ± 2.03 gr] to (W12) [176 ± 8.62 gr].  
 In Dex (3) (3000 + 3000 mg) mixture  
 (W7) [120 ± 27.01 gr] to  
 (W12) [186.51 ± 33.49 gr]  
 Weight of Male rats during injection of mixture extract.  
 In case of Male Control in Week (7) [156 ± 11.07 g]  
 to a significant increase week (12) [225 ± 23.14 g].  
 Male Control (Dexa)  
 Weight in Week (7) (123 ± 2.33 g) to a significant  
 increase week (12) (230.6 ± 29.92 g).  
 Dex (1) mixture administration 500 mg + 500 mg  
 In week (7) [151.2 ± 9.41 g] to week (12) [205.7 ± 30.4  
 g] significant increase.  
 In case of Dex (2) Mixture 1500 + 1500 mg  
 W (7) [154 ± 13.8 g] to W (12) [222 ± 14.4 g] another  
 elevation.  
 In case of Dex (3) Mixture 3000, 3000 mg  
 W (7) [132.2 ± 12.58 g] to W (12) [185.7 ± 13.91 g]  
 significant elevation of weights.

It may be concluded from the data presented in the  
 tables (1-12) that:

1. The weights of male rats were significantly  
 higher than female rats.
2. Mixture extract administration affects  
 significantly higher male rats as compared with  
 female rats.
3. The higher the content of the mixture of extract  
 of shrimp and oysters shell, the higher the weight  
 of rats recorded in males compared with females  
 rats.

### Discussion

The results in (Table 1-6) before mixture injections  
 reveals that in case of male rats and female rats the  
 following results:

In case of Tables (1-6) before the Mixture injections  
 (shrimp and oysters shells)

The weight of male and female rats follow the same  
 rhythm of gradual increase throughout the duration  
 from one to six weeks in different groups of male and  
 female rats control (1), Dexa (1), Dexa (2), Dexa (3)  
 and Dexa (4).

As for the Table (7-12) after the mixture injection  
 (Shrimp and oysters shells)

Male control significantly increase in weight of rats  
 from week seven to 12,

In case of Dexa (1) male control, there was a gradual  
 increase in weight of rats from week 7-12

In case of Dexa (2) male rats the increase of rats was  
 gradually from week 7-12, and higher than Dexa (1)  
 and Dexa (3)

In case of Dexa (3) there was a graded increase in  
 weight of male rats from week 7 to 12.

The weight of the Female rats from week 7 to 12 in  
 case of female control group increased significantly.

As for female control Dexa (4) the increase of weight  
 was significant from week 7 to 12

In case of Dexa (1) there was a significant increase in  
 weight from week 7 to 12

Dexa (2) female rats showed a significant increase in  
 weight from week 7 to 12

Dexa (3) female rats showed the higher increase in  
 body weight of female rats from 7 to 12 week with the  
 higher dose of shrimp and oyster mixture.

The results of the effect of the shrimp and oyster  
 mixture indicated that the mixture induce a significant  
 increase in weight gain of male and female rats  
 through the increase in duration (7 to 12 weeks) and  
 also through the increase in concentration of the  
 mixture and that male rats' weight was higher than  
 female rats.

Data presented in Table (1–6) (7–12) illustrate that in  
 cases of male rats compared to female rats, that shrimp  
 and oyster shell could significantly increase the body  
 weight and weight gain of the investigated rats. This  
 increased body weight and weight gain of the  
 investigated rats could be attributed to the presence of  
 shrimp and oyster shell rich in chitin and chitosan  
 together with protein and peptides that can affect the  
 weight of the rats in both direct and indirect ways. Also,  
 of importance to say, that oyster shell rich in calcium  
 carbonate and trace elements (zinc, selenium) induced  
 a slight increase in weight gain, as adequate calcium  
 intake has been associated with better metabolism and  
 may improve weight control in animals and human. In  
 addition, it can increase lean body mass (muscle and  
 bone) more than fat mass especially in growing  
 rats. (Al-Ali, (2021)

However, what determine the effect on weight gain  
 may be induced due to several factors depending on  
 dosage of chitosan and calcium that increase weight of  
 rats and increase lean body mass. Also of importance,  
 the duration of treatment, the longer duration allows  
 measurable body composition changes; other factors  
 may induced effects such as the form of extract whole  
 or purified, diet content such high fat diet, also health  
 state of the rats. (Abd El Rahman et al 2006)

In the same line, Mougios (2006) stated that protein  
 supplementations might stimulate body fibers and  
 muscle abilities affecting muscle functions and  
 decreasing muscle damage that led to induce extra  
 benefit toward boosting muscle health and function.  
 Wolfe (2000) added that hormones like growth  
 hormone, insulin growth factor 1 and testosterone  
 together with growth factors act a major role in muscle  
 growth and hypertrophy, also stem cells play a major  
 role in increasing muscle fibers and increasing lean  
 body mass in case of male compared with female  
 animals or human; and protein supplementation help  
 the process of growth, and weight gain, leading to

improve muscle function and strength in male compared with female.

Sadegh et al (2018) reported the important role of muscle stem cell (satellite cells), that are tissue residents stem cell that are required for the regenerative potential of healthy adult skeletal muscle tissue through expressing the paired box transcription factor PAX 7, leading to formation of muscle fibers, that lead to muscle growth and muscle strength and improve muscle force.

### Conclusion:

The results of this study about the effects of a mixture of shrimp peels extract and shellfish of some types of oysters on weight gain of experimental rats with osteoporosis induced with dexamethasone revealed the following conclusions:

1. That the mixture of shrimp peels and shell fish induced an elevated weight gain
2. The elevated weight gain of male rats were higher than the female rats
3. Dexamethasone may induce bad effect on weight gain in both male and female rats

### Recommendation

It is recommended the following:

- 1- Coordination with other osteoporosis treatments and medications to avoid health risks.
- 2- Physical activities must accompany supplements in cases of osteoporosis together with health education programs.
- 3- Eating appropriate amount of protein
- 4- The results may lead to the Bio-economic importance of shrimp and oyster shell due to the benefit of chitosan and chitin from the waste in agriculture and biotechnology industry, wastewater treatment, Energy conversion and biomedical material development

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