Research Article

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Cow milk and its dairy products ameliorate bone toxicity in the Coragen-induced rat model

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Abstract: Coragen is an insecticide that stimulates calcium release from intracellular stores of muscle cells causing death to sensitive species. The present study aimed to evaluate the bone toxic effect of Coragen and the potential therapeutic effect of cow milk, yogurt, and soft cheese in rats. Toxicity was induced by Coragen administration with different doses of 1/20 or 1/40 LD_{50} in rats. Groups of rats (n = 6) were treated with either 5 g milk, 5 g yogurt, or 1.5 g cheese. Coragen administration elevated alkaline and acid phosphatases activity and reduced the calcium and phosphorus level in urine and serum of rats administered with Coragen. Femur and tibia length, thickness, weight, and breaking force were decreased by Coragen administration and femur Ca and P contents as well. Bone mineral area (BMA), bone mineral content (BMC), bone mineral density (BMD), protein profile (total, albumin, and globulin), and antioxidant system (TAC, GSH, GP_X, GST, and SOD) were decreased by Coragen. All these parameters were improved on the treatment with milk and milk products. The results showed that yogurt treatment was significantly superior to the other treatments in increasing BMD (27%), breaking force (9%), femur Ca (41%), serum Ca (14%), and serum P (16%) and in reducing acid phosphatases (14%) and urine Ca and P by 8 and 10%, respectively. It can be concluded that the treatment with milk and milk products may provide treatment against osteoporosis and toxicity caused by Coragen.

Keywords: osteoporosis, Coragen, cow milk, yogurt, soft cheese

1 Introduction

Pesticides have long been used to improve the agricultural yield and control various pests [1,2]. Toxicological studies reported that pesticide exposure can alter the bone composition and that may lead to bone diseases such as osteoporosis [3–7]. Osteoporosis is a bone disease that causes bone density loss and increases the risk of bone fractures [5]. Chlorantraniliprole (trade name Coragen) is a new compound that belongs to a new class of selective insecticides (anthranilic diamides) and acts as a ryanodine receptor modulator. It stimulates the release of calcium from intracellular stores of muscle cells causing impaired muscle regulation, paralysis, and ultimately death of sensitive species. Coragen is used in agriculture against pests of the order Lepidoptera and Isoptera, also Diptera and Coleoptera species, in a wide variety of crops [8].

Pesticides can be toxic to other organisms such as birds, beneficial insects, fish, and soil microorganisms. Beneficial insects such as bees showed symptoms of apathy, slow movements, and lethargy after exposure to Coragen [9,10]. Coragen was highly toxic to fish such as *Channa punctatus* [11]. The fish (*Channa punctatus*) showed behavioral changes such as hyperactivity, erratic swimming, posture imbalance, and excess secretion of mucus overall the body surface after exposure to Coragen [11]. Many animal studies have reported that Coragen causes bodyweight reduction, elevation in liver enzyme activity, hemato-toxicity, and histopathological changes in liver, lung, and spleen [12–14]. Hassan et al. [15] reported that Coragen caused thrombocythemia, leukocytosis, microcytic anemia, kidney dysfunction, hyperuricemia, and elevated level of sex hormone and thyroid hormone in rats. Coragen is classified as a non-carcinogenic and non-toxic agent for humans; however, a 26-old woman had a cardiac manifestation after exposure to

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Coragen [16]. Exposure to Coragen has been reported to cause blood calcium reduction in rats [13]. Calcium is essential for cellular activation and responsible for bone rigidity [17]. Calcium deficiency is a key cause of osteoporosis [18]. Calcium reduction as a result of Coragen exposure may lead to bone loss. To the best of our knowledge, no previous research has yet investigated the effect of Coragen on bone mineralization or its potential toxic effect on bones.

Nutritional intervention may be a potential therapeutic approach to tackle Coragen toxicity. Milk and functional dairy products have been associated with health benefits of their constituents. Milk contains proteins, bioactive peptides, oligosaccharides, omega-3 fatty acids, conjugated linoleic acids, calcium, and vitamins. Fermented dairy products such as yogurt and soft cheese provide essential nutrients and probiotic bacteria [19,20]. Probiotics are live microorganisms that provide a health benefit to the host [21]. Products containing live probiotic bacteria have several health benefits such as blood cholesterol reduction and immunity improvement [22]. Also, it has been reported that milk and its functional dairy products have biological effects such as neuro-modulatory, immune-modulating, anti-inflammatory, anti-microbial, bone protective, and cardio-protective [23]. Milk and milk products have the antioxidant capacity and have the potential to protect against oxidative stress [23]. Skimmed milk (17%), yogurt (17%), and whey protein (6%) enhanced the bone mineral content and bone mineral density in ovariectomized rats [24]. Numerous *in vitro* studies showed that yogurt starter and probiotic lactobacilli can reduce pesticide load [25–28]. Probiotics showed an antioxidant, anti-inflammatory, and anti-fibrotic effect in ethephon-treated rats [29]. However, no studies have yet evaluated the effect of milk and milk products on pesticide toxicity.

In the light of the previously mentioned evidence and with the scarcity of data regarding the effect of Coragen on bone properties, the present study aimed to study the effect of Coragen on bone mineralization, bone mineral density, and biochemical parameters. This study also assessed the potential therapeutic effects of cow milk and milk products (yogurt and soft cheese) against the potential bone toxic effects of Coragen in rats.

2 Materials and methods

2.1 Chemical

Coragen 20% SC was obtained from the Central Agricultural Pesticide Laboratory (CAPL). The pesticide chlorantraniliprole with commercial name Coragen and IUPAC name is 3-bromo-*N*-[4-chloro-2-methyl-6-(methyl-carbamoyl)phenyl]-1-(3-chloro-2-pyridine-2-yl)-1*H*-pyrazole-5-carboxamide with structural formula of $C_{18}H_{14}BrCl_{12}N_5O_2$. Its chemical class is anthranilic diamide insecticide, and its LD_{50} >5,000 mg/kg body weight of male albino rats [30].

2.2 Preparation of yogurt and soft cheese and chemical analysis

2.2.1 Yogurt preparation

Cow milk samples were collected from the herds of the Faculty of Agriculture, Cairo University. The cow milk was heated up to 55°C, subsequently normalized and pasteurized at 71°C, then cooled to 40°C for the fermentation process. The starter culture for yogurt preparation was *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (Mecin Lab Faculty of Agriculture, Ain Shams University). The starter cultures were added and incubated according to the manufacturer's recommendations until pH 5.2 [31]. The samples were refrigerated at 4 ± 1°C.

2.2.2 Soft cheese preparation

Calcium chloride (0.01 g/5 L of milk) was added to warm cow milk (42°C) 30 min prior to the addition of the starter culture. Cooled milk was inoculated with a starter culture. Diluted liquid camel chymosin was added after inoculation of culture depending on the accomplishment of pH value. The milk was allowed to coagulate for 2 h after the addition of camel chymosin. The coagulated curd was cut and left to stand for 10 min and then was poured into a plastic mold lined with a cheesecloth, thereafter the whey was drained off from the curd. The cheese samples were collected in sterile containers and weighed immediately using a digital weighing balance, prior to storage in the refrigerator at 4 ± 1°C [32]. The weight of the cheese sample was recorded, and the yield of the cheese was calculated as follows:

$$\text{Cheese yield } (\%) = (\text{Weight of cheese})/(\text{Weight of milk}) \times 100.$$

2.2.3 Chemical analysis of milk and milk products

Different chemical parameters such as phosphorus content, total solids, moisture, ash, fat, protein, and lactose

in milk, yogurt, and soft cheese were estimated by the method described in AOAC [33]. Vitamins (A, C, D, E, B1, and B2) were determined according to procedures outlined in AOAC [33]. Mineral contents (Na, K, Ca, Mg, Fe, Zn, and Cu) were determined using atomic absorption spectrometry (Pye Unicum model SP 192 instrument) according to the method of Murthy and Rhea [34]. All samples were analyzed in triplicate.

2.3 Animal experiment

2.3.1 Diet and animals

A total of 54 male healthy Sprague-Dawley rats (150–160 g) were obtained from the animal house of the National Research Center, Dokki, Cairo, Egypt. The animals were housed under controlled environmental conditions (23 ± 1°C, 55 ± 5% humidity, and 12 h light: 12 h dark cycle). The animals were fed with a basal diet composed of 15% casein, 10% corn oil, 5% cellulose, 4% salt mixture, 1% vitamins mixture, and 65% starch. Food and water were given *ad-libitum* during the experimental period (90 days) [35].

2.3.2 Experimental design

The animals were fed on a basal diet for 14 days as an adaptation period. After the adaption period, rats were divided randomly into nine groups (six rats for each group). All the groups were fed on a basal diet. Group 1 (normal control group) was administered water orally three times per week. Group 2 (Coragen control group 1/20 LD_{50}) was orally administered with Coragen (1/20 LD_{50}) at a dose of 250 mg/kg body weight three times per week. Groups 3, 4, and 5 were orally administered with Coragen (1/20 LD_{50}) at a dose of 250 mg/kg body weight, and each of the three groups was treated with cow milk (5 g/kg), yogurt (5 g/kg), or soft cheese (1.5 g/kg), respectively, three times per week. Group 6 (Coragen control group 1/40 LD_{50}) was orally administered with Coragen (1/40 LD_{50}) at a dose of 125 mg/kg body weight three times per week. Groups 7, 8, and 9 were orally administered with Coragen (1/40 LD_{50}) at the same dose of group 6, and each group was treated with cow milk (5 g/kg), yogurt (5 g/kg), or soft cheese (1.5 g/kg), respectively, three times per week for 90 days. At the end of the experiment, body weight was recorded and 24 h urine samples were collected for mineral content determination using standard methods. Blood samples were obtained from fasted, anesthetized rats, and serum was separated for the estimation of elements content (Na, K, Ca, Mg, and P), protein profile (total, albumin, globulin) content, reduced glutathione (GSH), total antioxidant capacity, phosphatases (acid and alkaline) activity, glutathione peroxidase activity (GP_X), glutathione-*S*-transferase activity (GST), and superoxide dismutase activity (SOD) according to the methods of Gregor et al. [36], Bergmeyer et al. [37], Kind and King [38], Koracevic et al. [39], Belfield and Golberg [40], Rotruck et al. [41], Grant and Matsumura [42], and Kakkar et al. [43], respectively. The biochemical test kits were obtained from Bio-diagnostic Company (Cairo, Egypt). The right femur and tibia of bones were separated, cleaned, and weighed. The length and thickness of the femur and tibia were measured using an ABS digimatic solar caliper (Tri-State Instrument Service, Fort Wayne, TX) [44]. The breaking force of the femur and tibia was measured using the Digital Force Gauge model, FGN-50, Japan [45]. The bone mineral parameters were measured by using a dual-X-ray absorptiometry (DXA) model, Norland XRE-46 [44].

Ethical approval: The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals, and has been approved by the Ethics Committee of the Cairo University, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.4 Statistical analysis

Statistical analysis was performed with SPSS software (version 17). Values are expressed as mean ± standard error (SE). Comparisons between groups were performed with one-way analysis of variance (ANOVA) followed by Tukey's test. *P* values were compared for all experimental groups, and $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 Chemical composition of cow milk and its products

Cow milk and its products (yogurt and soft cheese) have an important role in human nutrition. The compositions

of cow milk, yogurt, and soft cheese are summarized in Table 1. The result showed that milk and its products had good nutritive essential constituents. Milk and yogurt were comparable in their content of protein, fat, lactose, ash, and moisture. The protein and fat content of soft cheese were higher than milk and yogurt. Soft cheese mineral content (Na, Ca, Mg, Fe, Zn, and Cu) was significantly higher than milk and yogurt except for potassium content was higher in yogurt. The yield of soft cheese was 30% from cow milk.

3.2 Mineral content and osteoporosis evaluation

K, Mg, Ca, and P contents were determined in serum and urine of rats (Table 2). Serum and urine contents of K and Mg were not affected by the administration of Coragen (1/20 LD_{50} or 1/40 LD_{50}). However, Coragen (1/20 or 1/40 LD_{50}) administration significantly decreased the level of Ca and P in serum and significantly increased their level in urine when compared with normal control rats. Treatments with cow milk, yogurt, and soft cheese ameliorated the harmful effect of Coragen. Milk and milk products significantly increased the concentration of Ca in the serum of rats administered with Coragen at a dose of 1/20 LD_{50}. Only yogurt and soft cheese treatments significantly increased serum Ca and P levels in Coragen-induced rats with a dose of 1/40 LD_{50}. However, the curative effect of yogurt was more effective than cow milk and soft cheese treatments on the reduction of Ca and P in the urine of rats administered with Coragen at a dose of 1/20 LD_{50}.

Table 1: Chemical composition of cow milk, yogurt, and soft cheese

	Milk	Yogurt	Soft cheese
Total solids (%)	12.5 ± 1.02b	13.1 ± 1.00b	46.0 ± 2.88a
Water (%)	87 ± 6.66a	87 ± 5.97a	53.0 ± 4.01b
Protein (%)	3.3 ± 0.27b	3.4 ± 0.21b	16.3 ± 1.02a
Lactose (%)	4.6 ± 0.31ab	5.5 ± 0.33a	3.2 ± 0.22b
Fat (%)	3.7 ± 0.24b	3.2 ± 0.19b	19.8 ± 1.12a
Ash (%)	0.8 ± 0.04b	0.9 ± 0.06b	7.3 ± 0.46a
Na (mg/L)	37 ± 2.41b	40 ± 2.12b	2769 ± 109a
K (mg/L)	102 ± 9.21b	1080 ± 7.11a	115 ± 7.21b
Ca (mg/L)	110 ± 8.71b	118 ± 3.12b	456 ± 21.72a
P (mg/L)	90 ± 4.01b	101 ± 6.26b	273 ± 15.55a
Mg (mg/L)	13.10 ± 1.00b	16 ± 1.00b	45 ± 2.76a
Fe (µg/mL)	0.35 ± 0.02a	0.2 ± 0.01b	0.2 ± 0.01b
Zn (µg/mL)	1.30 ± 0.07b	0.2 ± 0.01c	1.9 ± 0.10a
Cu (µg/mL)	0.10 ± 0.01b	0.12 ± 0.01b	0.31 ± 0.02a
Vit. A (mg/mL)	0.36 ± 0.02b	0.24 ± 0.01c	0.70 ± 0.04a
Vit. C (mg/mL)	5.0 ± 0.33a	0.11 ± 0.01b	0.02 ± 0.001c
Vit. B1 (mg/mL)	0.02 ± 0.001c	0.06 ± 0.004b	0.08 ± 0.005a
Vit. B2 (mg/mL)	0.04 ± 0.02c	0.18 ± 0.01b	0.37 ± 0.02a
Vit. D (mg/mL)	0.08 ± 0.05b	0.10 ± 0.01b	0.15 ± 0.01a
Vit. E (mg/mL)	0.10 ± 0.01b	0.09 ± 0.01b	0.13 ± 0.01a

Values are mean ± SE; the same letter in each row is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

The result showed that administration of Coragen at a dose of either 1/20 or 1/40 LD_{50} significantly decreased the length, thickness, and weight of femur and tibia when compared with normal control rats (Table 3). The treatments with cow milk, yogurt, and soft cheese significantly increased the length and weight of femur in Coragen-induced rats (1/20 LD_{50} or 1/40 LD_{50}). Only yogurt treatment significantly increased the thickness of femur in the Coragen (1/40 LD_{50}) administration group. The thickness of the tibia significantly increased after treatment of milk, yogurt, and cheese for both doses of Coragen-administered groups. In addition, yogurt treatment significantly increased the length of the tibia in the Coragen-administered (1/20 LD_{50}) group. The result showed that yogurt treatment was superior to the other treatments.

Administration of Coragen significantly decreased the bone strength of the femur and tibia when compared with normal control rats (Table 4). Cow milk and its products increased the breaking force in Coragen-induced rats at a dose of 1/40 LD_{50}. However, only yogurt significantly increased the breaking force of femur and tibia in Coragen-induced rats at a dose of 1/20 LD_{50}. Coragen significantly reduced femur Ca and P content when compared with normal control rats (Table 4). Milk, yogurt, and soft cheese treatments significantly increased Ca content in the femur of Coragen-induced rats with both doses but all treatments did not affect femur P content.

Bone mineral area (BMA), bone mineral content (BMC), and bone mineral density (BMD) of the total, proximal, and distal bone were presented in Table 5. The total, proximal, and distal bone parameters (BMA, BMC, and BMD) were significantly decreased in Coragen-administered rats when compared with normal control rats. Treatment with milk and its products reversed the

Table 2: Effect of milk, yogurt, and soft cheese on mineral concentration in serum and urine of Coragen-induced rats

	Serum (mg/dL)				Urine (mg/dL)			
	K	Mg	Ca	P	K	Mg	Ca	P
G1 (control)	18.08 ± 1.34a	10.34 ± 0.80a	10.44 ± 0.78a	25.21 ± 1.42a	118.03 ± 6.66a	11.00 ± 0.61a	10.66 ± 0.61c	101.11 ± 6.61c
G2 (control 1/20 LD_{50} Coragen)	19.01 ± 1.73a	11.00 ± 0.94a	8.01 ± 0.66c	19.76 ± 1.37b	126.61 ± 9.14a	10.98 ± 0.54a	12.76 ± 0.77a	124.02 ± 7.23a
G3 (1/20 LD_{50} Coragen + 5 g milk)	18.77 ± 1.62a	10.43 ± 0.78a	9.10 ± 0.72b	21.01 ± 1.72b	122.71 ± 7.16a	11.01 ± 0.73a	12.00 ± 0.69ab	119.47 ± 6.41a
G4 (1/20 LD_{50} Coragen + 5 g yogurt)	18.00 ± 1.77a	10.66 ± 0.89a	9.71 ± 0.74a	23.21 ± 1.66a	119.99 ± 7.18a	10.87 ± 0.81a	11.66 ± 0.59b	111.51 ± 7.77b
G5 (1/20 LD_{50} Coragen + 5 g cheese)	17.97 ± 1.62a	10.74 ± 0.69a	9.65 ± 0.76a	22.22 ± 1.74ab	120.27 ± 8.74a	10.98 ± 0.98a	11.81 ± 0.57ab	115.07 ± 6.97ab
G6 (control 1/40 LD_{50} Coragen)	18.61 ± 1.41a	10.81 ± 0.94a	8.49 ± 0.69b	20.16 ± 1.59b	121.27 ± 6.21a	11.00 ± 0087a	12.16 ± 0.81ab	115.61 ± 8.00ab
G7 (1/40 LD_{50} Coragen + 5 g milk)	18.12 ± 1.21a	11.00 ± 0.59a	9.34 ± 0.80b	22.00 ± 1.56ab	119.71 ± 6.21a	10.26 ± 0.78a	11.21 ± 0.69bc	109.00 ± 6.21bc
G8 (1/40 LD_{50} Coragen + 5 g yogurt)	18.00 ± 1.32a	10.61 ± 0.71a	9.91 ± 0.82a	23.99 ± 1.99a	120.11 ± 7.14a	10.99 ± 0.91a	11.2 ± 0.69bc	109.00 ± 6.21bc
G9 (1/40 LD_{50} Coragen + 5 g cheese)	18.21 ± 1.41a	10.43 ± 0.82a	9.82 ± 0.82a	23.12 ± 2.00a	112.00 ± 6.21a	11.10 ± 0.72a	11.47 ± 0.62bc	110.52 ± 5.91bc

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

Table 3: Femur and tibia length, thickness, and weight of the different experimental groups

	Femur bone			Tibia bone		
	Length (mm)	Thickness (mm)	Weight (g)	Length (mm)	Thickness (mm)	Weight (g)
G1 (control)	18.55 ± 1.11a	1.68 ± 0.090a	0.51 ± 0.032a	25.21 ± 1.76a	1.11 ± 0.063a	0.44 ± 0.033a
G2 (control 1/20 LD_{50} Coragen)	15.20 ± 0.99b	1.36 ± 0.071b	0.28 ± 0.022e	19.12 ± 1.47c	0.71 ± 0.042d	0.30 ± 0.021b
G3 (1/20 LD_{50} Coragen + 5 g milk)	17.23 ± 1.00a	1.47 ± 0.081ab	0.34 ± 0.021d	20.67 ± 1.84bc	0.84 ± 0.056b	0.36 ± 0.027b
G4 (1/20 LD_{50} Coragen + 5 g yogurt)	17.88 ± 1.03a	1.52 ± 0.081ab	0.40 ± 0.031c	21.72 ± 1.53b	0.89 ± 0.056b	0.37 ± 0.030ab
G5 (1/20 LD_{50} Coragen + 5 g cheese)	17.37 ± 1.01a	1.48 ± 0.090b	0.35 ± 0.032d	20.71 ± 1.62bc	0.85 ± 0.055bc	0.35 ± 0.028b
G6 (control 1/40 LD_{50} Coragen)	16.88 ± 0.97b	1.42 ± 0.071b	0.31 ± 0.023d	20.31 ± 1.50bc	0.82 ± 0.053c	0.36 ± 0.025b
G7 (1/40 LD_{50} Coragen + 5 g milk)	17.90 ± 0.98a	1.50 ± 0.082ab	0.40 ± 0.032c	22.00 ± 2.00b	0.90 ± 0.051b	0.38 ± 0.030ab
G8 (1/40 LD_{50} Coragen + 5 g yogurt)	18.01 ± 1.01a	1.58 ± 0.101a	0.45 ± 0.030b	22.61 ± 1.87ab	0.96 ± 0.067b	0.40 ± 0.030ab
G9 (1/40 LD_{50} Coragen + 5 g cheese)	17.87 ± 1.00a	1.49 ± 0.081ab	0.41 ± 0.021bc	21.97 ± 1.79b	0.91 ± 0.071b	0.38 ± 0.029ab

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

Table 4: Femur and tibia breaking force and femur mineral content of different experimental groups

	Breaking force		Femur mineral content	
	Femur (N)	Tibia (N)	Calcium (g/100 g)	Phosphorus (g/100 g)
G1 (control)	105.5 ± 9.74a	81.32 ± 5.12a	95.27 ± 5.47a	11.00 ± 0.62a
G2 (control 1/20 LD_{50} Coragen)	71.17 ± 4.13d	65.47 ± 4.34b	49.74 ± 3.24c	8.91 ± 0.54b
G3 (1/20 LD_{50} Coragen + 5 g milk)	76.00 ± 4.11cd	69.01 ± 5.00b	78.71 ± 4.26b	9.46 ± 0.71b
G4 (1/20 LD_{50} Coragen + 5 g yogurt)	78.42 ± 3.99c	71.32 ± 5.27ab	85.71 ± 5.71ab	10.01 ± 0.58ab
G5 (1/20 LD_{50} Coragen + 5 g cheese)	75.89 ± 5.01cd	69.34 ± 4.99b	77.77 ± 4.27b	10.23 ± 0.57ab
G6 (control 1/40 LD_{50} Coragen)	76.89 ± 4.74cd	68.42 ± 5.01b	51.11 ± 3.11c	10.12 ± 0.60ab
G7 (1/40 LD_{50} Coragen + 5 g milk)	85.12 ± 4.44b	71.62 ± 5.24ab	81.12 ± 5.12b	10.25 ± 0.58ab
G8 (1/40 LD_{50} Coragen + 5 g yogurt)	90.14 ± 6.16b	74.31 ± 5.46ab	87.22 ± 5.22ab	10.56 ± 0.61a
G9 (1/40 LD_{50} Coragen + 5 g cheese)	86.00 ± 5.67b	72.01 ± 5.96ab	81.78 ± 5.55b	10.78 ± 0.71a

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

reduction of total BMA, BMC, and BMD in Coragen-induced rats (1/20 LD_{50}). Only yogurt significantly altered the total BMD in Coragen-induced rats (1/40 LD_{50}). All treatments significantly increased distal bone BMC and BMD in either Coragen-induced rats at a dose of 1/20 LD_{50} or Coragen-induced rats at a dose of 1/40 LD_{50}. Distal bone BMA was significantly increased after treatment with milk and its products in Coragen-induced rats at a dose of 1/20 LD_{50}. However, only yogurt significantly elevated distal bone BMA in Coragen-induced rats at a dose of 1/40 LD_{50}. Proximal bone BMC was significantly improved after treatment with milk and its products in both doses of Coragen-induced rats. Cow milk, yogurt, and cheese significantly elevated the proximal bone BMA in Coragen-induced rats at a dose of 1/20 LD_{50}. Proximal bone BMD was insignificantly improved by treatments.

3.3 Protein profile and phosphatase activity

Serum total protein, albumin, and globulin contents were altered by Coragen ingestion (Table 6). The changed values of total proteins, albumin, and globulin showed a significant decrease either by 1/20 LD_{50} or by 1/40 LD_{50} of Coragen ingestion, but 1/20 LD_{50} was more effective than 1/40 LD_{50}. Treatments with cow milk and its products (yogurt and cheese) significantly attenuated the harmful effect of Coragen (1/20 LD_{50} or 1/40 LD_{50}) on protein profile and globulin content in serum and improved these disturbances. Serum albumin was significantly increased after treatment with milk, yogurt, and cheese in Coragen-induced rats (1/40 LD_{50}).

Coragen ingestion caused a highly significant stimulation in ALP and ACP activity when compared with normal control rats (Table 6). The influence of Coragen 1/20 LD_{50} on ALP and ACP activities was more than that of Coragen 1/40 LD_{50}. In addition, the results showed that the levels of ALP and ACP activity were improved upon treatment with cow milk and its products (yogurt and cheese). However, the treatment with yogurt was superior.

3.4 The antioxidant system

The effects of Coragen toxicity on the total antioxidant capacity (TAC), glutathione (GSH), and antioxidant enzyme activity were investigated (Table 7). Administering Coragen at a dose of 1/20 LD_{50} and 1/40 LD_{50} significantly decreased serum TAC level, GSH, and antioxidant enzyme activity (GST, $GP_{X,}$ and SOD). Yogurt treatment led to a significant elevation of GSH and TAC level and antioxidant enzyme activity in rats induced by Coragen at a dose of 1/20 LD_{50}. However, milk and soft cheese ameliorated the harmful effect of Coragen but not to a significant level.

4 Discussion

Osteoporosis is a bone metabolic disease characterized by bone mineral density reduction and bone microstructure degradation, which can increase bone fragility and fracture risk [46,47]. Toxicological studies reported that exposure to pesticides, such as organochlorine, can alter bone mineralization and composition and may lead to osteoporosis [3–7]. Chlorantraniliprole (the active ingredient of Coragen) is a ryanodine receptor activator and

Table 5: Total, proximal, and distal bone mineral area (BMA), bone mineral content (BMC), and bone mineral density (BMD) of different experimental groups

	Total			Proximal bone			Distal bone		
	BMA (cm^2)	**BMC (g)**	**BMD (g/cm^3)**	**BMA (cm^2)**	**BMC (g)**	**BMD (g/cm^3)**	**BMA (cm^2)**	**BMC (g)**	**BMD (g/cm^3)**
G1 (control)	2.34 ± 0.161a	0.224 ± 0.013a	0.120 ± 0.008a	0.615 ± 0.041a	0.161 ± 0.010a	0.099 ± 0.006a	0.653 ± 0.041a	0.163 ± 0.009a	0.199 ± 0.011a
G2 (control 1/20 LD_{50} Coragen)	1.06 ± 0.072d	0.099 ± 0.006d	0.012 ± 0.003d	0.411 ± 0.027d	0.045 ± 0.003d	0.080 ± 0.006b	0.410 ± 0.030d	0.040 ± 0.003d	0.088 ± 0.005c
G3 (1/20 LD_{50} Coragen + 5 g milk)	1.62 ± 0.100bc	0.164 ± 0.007bc	0.051 ± 0.003c	0.469 ± 0.030c	0.089 ± 0.005c	0.085 ± 0.007b	0.483 ± 0.032c	0.072 ± 0.004c	0.121 ± 0.007d
G4 (1/20 LD_{50} Coragen + 5 g yogurt)	1.88 ± 0.100bc	0.185 ± 0.011b	0.060 ± 0.004bc	0.502 ± 0.032b	0.099 ± 0.006bc	0.098 ± 0.007ab	0.500 ± 0.033c	0.080 ± 0.005bc	0.147 ± 0.008b
G5 (1/20 LD_{50} Coragen + 5 g cheese)	1.60 ± 0.099bc	0.159 ± 0.010c	0.050 ± 0.003c	0.472 ± 0.028bc	0.090 ± 0.006c	0.086 ± 0.005b	0.490 ± 0.032c	0.071 ± 0.006c	0.119 ± 0.006d
G6 (control 1/40 LD_{50} Coragen)	1.71 ± 0.101bc	0.168 ± 0.012bc	0.051 ± 0.004c	0.481 ± 0.031bc	0.051 ± 0.003d	0.088 ± 0.006b	0.489 ± 0.040c	0.051 ± 0.003d	0.100 ± 0.006e
G7 (1/40 LD_{50} Coragen + 5 g milk)	1.82 ± 0.113bc	0.180 ± 0.012b	0.061 ± 0.005bc	0.500 ± 0.040ab	0.099 ± 0.007bc	0.090 ± 0.007ab	0.502 ± 0.041c	0.081 ± 0.005bc	0.148 ± 0.009b
G8 (1/40 LD_{50} Coragen + 5 g yogurt)	2.00 ± 0.0103bc	0.189 ± 0.012b	0.070 ± 0.004b	0.589 ± 0.044a	01089 ± 0.008b	0.094 ± 0.007a	0.567 ± 0.039b	0.098 ± 0.006b	0.161 ± 0.010b
G9 (1/40 LD_{50} Coragen + 5 g cheese)	1.87 ± 0.111bc	0.179 ± 0.011b	0.062 ± 0.004bc	0.501 ± 0.039b	0.100 ± 0.007bc	0.090 ± 0.006ab	0.501 ± 0.032c	0.082 ± 0.005bc	0.150 ± 0.009b

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

Table 6: Protein profile and phosphatase activity of different experimental groups

	Protein profile			Phosphatases activity	
	Total protein (g/dL)	**Albumin (g/dL)**	**Globulin (g/dL)**	**ACP (U/L/mg protein)**	**ALP (U/L/mg protein)**
G1 (control)	6.76 ± 0.41a	4.50 ± 0.31a	2.26 ± 0.17a	46.12 ± 3.33a	81.07 ± 5.12e
G2 (control 1/20 LD_{50} Coragen)	4.10 ± 0.29a	2.89 ± 0.20c	1.21 ± 0.10d	60.11 ± 4.12c	150.11 ± 7.78a
G3 (1/20 LD_{50} Coragen + 5 g milk)	5.11 ± 0.32bc	3.18 ± 0.21bc	1.93 ± 0.09b	56.00 ± 3.87bc	100.21 ± 6.24c
G4 (1/20 LD_{50} Coragen + 5 g yogurt)	5.20 ± 0.31bc	3.42 ± 0.19bc	1.78 ± 0.06bc	51.11 ± 3.11b	91.61 ± 6.61d
G5 (1/20 LD_{50} Coragen + 5 g cheese)	5.13 ± 0.28bc	3.20 ± 0.22bc	1.93 ± 0.07b	55.16 ± 3.22bc	99.71 ± 7.12c
G6 (control 1/40 LD_{50} Coragen)	4.68 ± 0.31c	3.10 ± 0.18c	1.58 ± 0.08c	55.51 ± 4.00c	141.11 ± 8.82b
G7 (1/40 LD_{50} Coragen + 5 g milk)	5.41 ± 0.32b	3.61 ± 0.18b	1.80 ± 0.08b	52.14 ± 3.27bc	94.71 ± 5.41cd
G8 (1/40 LD_{50} Coragen + 5 g yogurt)	5.62 ± 0.40b	3.72 ± 0.27b	1.90 ± 0.10b	50.11 ± 3.27b	90.00 ± 5.55d
G9 (1/40 LD_{50} Coragen + 5 g cheese)	5.44 ± 0.36b	3.66 ± 0.26b	1.78 ± 0.11bc	53.00 ± 4.00bc	95.00 ± 5.61d

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

Table 7: The antioxidant capacity and activity of different experimental groups

	Antioxidant				
	Total antioxidant capacity (mM/L)	**GSH (mM/mL)**	**GST activity (mM/mL)**	**GP_X activity (U/L)**	**SOD activity (U/L)**
G1 (control)	1.75 ± 0.09a	0.52 ± 0.031a	53.27 ± 3.71a	920.516 ± 50.11a	351 ± 20.1a
G2 (control 1/20 LD_{50} Coragen)	1.50 ± 0.10b	0.38 ± 0.020c	46.72 ± 2.94b	785.11 ± 41.11b	262 ± 18.3c
G3 (1/20 LD_{50} Coragen + 5 g milk)	1.60 ± 0.07ab	0.42 ± 0.023bc	48.66 ± 3.00ab	812.03 ± 51.03ab	298 ± 19.4b
G4 (1/20 LD_{50} Coragen + 5 g yogurt)	1.67 ± 0.09a	0.44 ± 0.030b	50.97 ± 2.94a	851.21 ± 43.94a	300 ± 20.2b
G5 (1/20 LD_{50} Coragen + 5 g cheese)	1.62 ± 0.08a	0.42 ± 0.026bc	48.71 ± 2.48ab	801.11 ± 52.22ab	295 ± 18.9b
G6 (control 1/40 LD_{50} Coragen)	1.54 ± 0.08b	0.40 ± 0.028bc	49.88 ± 3.01ab	800.21 ± 50.31ab	284 ± 18.8bc
G7 (1/40 LD_{50} Coragen + 5 g milk)	1.68 ± 0.09ab	0.46 ± 0.029b	50.27 ± 3.11ab	842.00 ± 42.48ab	317 ± 20.4ab
G8 (1/40 LD_{50} Coragen + 5 g yogurt)	1.70 ± 0.07ab	0.48 ± 0.031ab	51.34 ± 3.12ab	867.77 ± 49.99a	321 ± 21.0ab
G9 (1/40 LD_{50} Coragen + 5 g cheese)	1.67 ± 0.07ab	0.44 ± 0.030b	50.32 ± 3.23ab	851.21 ± 54.21ab	311 ± 22.10ab

Values are mean ± SE; the same letter in each column is not significantly different, and different letters are significantly different at the level of 0.05 probability levels.

controls the release of calcium from intracellular stores in insects [8]. The flow of calcium is regulated by ryanodine receptors, which mediate several physiological cellular processes such as skeletal muscle excitation-contraction coupling process, neurotransmission, neurohormones release, and cardiac contraction [48]. In our previous study, Coragen with different doses reduced serum calcium in rats [13]. There is no report available regarding the possibility of bone toxicity and osteoporosis after prolonged exposure to Coragen in rats. Therefore, this study evaluated the effect of Coragen at two different doses and assessed the potential ameliorative effect of milk and milk products.

Bone is the main component of the skeletal system and consists of 50–70% of minerals, 20–40% of organic matter, and 5–10% of water. The bone functions are locomotion, bone marrow protection, and storage of calcium and phosphate. Calcium and phosphate are key components for hydroxylapatite which is an essential mineral compound in normal bone and responsible for the rigidity of bones [17]. When the calcium circulation level decreases after calcium elimination from the body through urination, parathyroid hormone is activated causing increased bone turnover [49]. Blood calcium deficiency is associated with the risk of osteoporosis [46]. Thus, calcium and phosphorus intake is important for healthy bones and normal BMD. The high dietary ratio of Ca/P has a positive effect on bone mass [50]. Dairy products are considered the best dietary source of calcium due to their high calcium content and high absorption rate [51]. Cow milk and its products (yogurt and soft cheese) have higher calcium content than camel and buffalo milk and considerable amounts of phosphorus and vitamin A and D more than camel and buffalo milk [52]. Numerous clinical studies on dairy products and calcium supplementation in children reported that dairy products and calcium have a beneficial effect on bone mineral mass during growth [53,54]. Bone mineral density and bone strength were increased after treatment with cheese fortified with calcium in rats [55]. Bovine milk provided a positive effect on bone strength, bone length, and bone mineralization in rats [56]. Dried yogurt supplemented with chicory increased the strength of bones and bone calcium concentration in calcium-deficient rats [57]. In the current study, the reduction of calcium and phosphorus levels in blood and bones by Coragen was reversed by treatment with milk products. Calcium content reduction was associated with a reduction in breaking force, total, proximal, and distal BMA, BMC, and BMD in Coragen intoxicated control groups. Treatment with milk, yogurt, and soft cheese exhibited a positive effect on bone characters (BMA, BMC and BMD) and breaking force.

Yogurt was the best treatment to protect against bone loss, which may be due to its richness in probiotics. Probiotics produce short-chain fatty acids, which decrease the pH of the intestinal tract, consequently improving intestinal calcium absorption and may prevent or decrease bone loss and restore the decreased levels of plasma Ca [58]. Studies reported that some strains of probiotics (*Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Bifidobacterium longum*) had a positive influence on osteoporosis [58–60]. Osteoblasts and osteoclasts cells are responsible for bone formation and bone resorption, respectively, and both influence bone density. When osteoplastic bone resorption rate becomes higher than osteoplastic bone formation rate, bone mass reduces and osteoporosis occurs [61]. Probiotics affect osteoblasts and osteoclasts cells during the process of bone remodeling [58]. *Lactobacillus casei* 393 from fermented milk improved BMD reduction in ovariectomized rats and increased bone strength [59]. Moreover, probiotics synthesize vitamins such as vitamin K, D, C, and folate which are essential for bone formation and growth [58]. There is some evidence suggesting that *Lactobacillus plantarum* has degradation potential toward organophosphate pesticides [27,62,63].

Alkaline phosphatase and acid phosphatase are markers for bone formation and bone resorption, respectively [64]. ALP and ACP activity were significantly increased in Coragen-induced rats when compared with normal control rats. Dutta et al. [12] reported that Coragen increased the level of alkaline phosphatase and that induction was reduced by *Pterocarpus santalinus* treatment in rats. The elevated ALP and ACP activity could contribute to a high bone turnover rate, through an elevation in bone formation and resorption, with bone resorption usually higher than bone formation which may cause bone loss [65]. The positive role of milk and milk products supplemented diets was observed in the present study through the improvement in bone metabolic markers ALP and ACP activity. Hypoproteinemia is associated with hypocalcemia [66]. There is a positive correlation between albumin/globulin ratio and bone mineral density [67]. Al-Aqaby et al. [68] reported that total protein, albumin, and globulin levels increased after treatment with milk supplemented with probiotics. In the present study, protein profile (total, albumin, and globulin) was decreased by Coragen. Treatment with milk, yogurt, and soft cheese reversed that reduction. The alteration of serum total protein and protein fractions level (albumin and globulin) resulted in parallel changes in serum calcium level in the present study.

One of many possible underling mechanisms of pesticide toxicity is oxidative stress production. Coragen

administration has been found to cause oxidative stress and alteration of the antioxidant defense system [12–14]. Oxidative stress occurs by an imbalance between the antioxidant defense system and the production of free radicals and that can lead to tissue damage and numerous pathological conditions. The antioxidant defense system (enzymatic and non-enzymatic) is scavenging various reactive oxygen species and free radicals by different mechanisms [69]. SOD, catalase, GP_X, and GST enzymes are considered the first line of defense during the reactive oxygen species scavenging process and maintain the balance between the antioxidant defense system and the production of free radicals [69]. SOD catalyzes the dismutation of superoxide radicals to oxygen and hydrogen peroxide; hydrogen peroxide, in turn, is converted by catalase to oxygen and water. GP_X is an antioxidant enzyme that plays a vital role in the reduction of hydrogen peroxide by holding the status of a redox system (GSH/GSSG) in the nonenzymatic antioxidant GSH system. Glutathione transferase has several biological roles including cell protection against xenobiotics and oxidative stress [69]. In the present study, the significant reduction in total antioxidant capacity, GSH level, and the antioxidant enzyme activity of SOD, GP_X, and GST due to exposure to Coragen (1/20 LD_{50} and 1/40 LD_{50} doses) for a prolonged time suggests the onset of Coragen-induced oxidative stress and free radical production in rats. Yogurt treatment was superior in increasing the antioxidant defense system in rats induced by Coragen at a dose of 1/20 LD_{50}. Yogurt antioxidant efficacy may be attributed to its probiotic and prebiotic content which is usually more than milk or soft cheese. *In vitro* and *in vivo* studies reported that lactic acid bacteria and yogurt supplementation modulate free radical production by reducing the oxidative stress marker level and increasing antioxidant enzyme activity [70–72]. *Lactobacillus acidophilus* increased the total antioxidant capacity in pesticides-induced rats [29]. Some studies showed that dried plums rich in antioxidant agents had a positive effect on the whole body and spine BMD, and the trabecular bone [73].

5 Conclusion

Coragen ingestion had negative effects on calcium and bone characters leading to osteoporosis as a result of BMD reduction. Moreover, Coragen ingestion showed bone osteoclasts activity higher than bone osteoblasts activity because of ALP and ACP activity alteration. Treatment with milk, yogurt, and soft cheese attenuated the disturbing effects of Coragen toxicity. These desirable influences of cow milk and its products varied with the different products. Yogurt treatment resulted in the highest improvement for the studied parameters of intoxicated animals. Several essential nutrients and different components are provided by milk and functional dairy products. Yogurt was superior to milk and soft cheese treatments, which may be due to the high prebiotic and probiotic content. Adding milk and milk products to the diet may protect against the toxicological effects of Coragen.

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