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High-Fat Diet Induced Bone Defects in Rats, and The Ameliorative Effect of *Portulaca oleracea* and *Cucurbita moschata* Seeds, and Their Phytochemical Characteristics

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Abstract

In the current study, the negative impact of a high-fat diet and obesity on bone health was investigated. The protective effect of *Portulaca oleracea* (purslane), *Cucurbita moschata* (pumpkin) seeds, and a mix of them, as well as their efficiency in treating bone damage caused by obesity, was estimated. Bakery products (cake, toast, and crackers) fortified with purslane, pumpkin seeds, and a combination of both were prepared and evaluated for their chemical and sensory properties. Phytochemical characterizations of the tested seeds were evaluated. Vitamin and amino acid contents in the tested seeds were estimated using HPLC analysis. The results revealed that cakes fortified with pumpkin seed powder, toast supplemented with purslane seed flour, and crackers made with a mixture of pumpkin and purslane seed flour showed the highest acceptability (8.9, 8.2, and 7.7, respectively). All fortified products showed a markedly high content of fiber and ash. Histopathological findings revealed that a high-fat diet caused marked degeneration of hepatocytes, thinning of compact bone, and loose trabeculae. It is worth mentioning that nearly normal bone shafts with almost no defects are observed in the pumpkin and mixed feeding groups (prevention and treatment), as well as in the purslane treatment group. Furthermore, pumpkin and purslane seeds are rich in a diversity of phytoconstituents. Glutamic acid is the major amino acid in purslane and pumpkin seeds. Moreover, vitamin B3 was the predominant vitamin in purslane seeds, while vitamins B1 and B6 were the predominant vitamins in pumpkin seeds. The results revealed that the tested seeds are rich in minerals, with potassium and iron being the most predominant minerals. These nutrients are associated with the observed improvement in the histopathological findings. Our results highlight the efficacy of purslane and pumpkin seed in mitigating the harmful effects that may occur on bones as a consequence of high-fat feeding or obesity.

Keywords: Bakery products; bone defects; obesity; pumpkin seeds; purslane seeds.

1. Introduction

Several key nutrients are essential for maintaining strong and healthy bones. These include calcium, vitamin D, vitamin K, protein, magnesium, phosphorus, and potassium. A balanced diet rich in these nutrients, combined with regular exercise, can significantly contribute to maintaining bone health throughout life. Additionally, other essential nutrients, such as zinc and copper, also play a crucial role in bone health by facilitating the binding of minerals to the protein structure of the bone. Bone tissue cells (osteoblasts) and adipose tissue cells (adipocytes) originate from the same precursor stem cells. Their metabolic interactions influence bone tissue homeostasis [1]. High-fat intake is associated with numerous harmful diseases, and both the quantity and quality of fat influence bone health, as excess saturated fat negatively impacts bone mineral density (BMD) [2].

A high-fat diet leads to obesity, which increases the probability of bone fractures [3]. When combined with estrogen deficiency, this has deleterious effects, decreasing bone mass and increasing bone fragility. Arthritis is also linked to a high-fat diet and obesity [4].

Pumpkin (*Cucurbita moschata*), a member of the Cucurbitaceae family, is a nutrient-dense food rich in diverse phytochemicals. Pumpkin and its seeds are rich in nutrients needed for promoting bone health, such as minerals (calcium, potassium, zinc, iron, phosphorus, and magnesium). It also contains vitamins such as vitamin K and D. Pumpkin seeds are rich in dietary fiber, which enhances health and helps treat many chronic diseases [5, 6]. Pumpkin seed extract showed an ameliorating effect on osteoporosis and aging-related diseases [7]. Pumpkin can help decrease oxidative stress, which can have harmful effects on bone tissue [1].

Purslane (*Portulaca oleracea* L.) belongs to the Portulacaceae family. Purslane seeds contain numerous bioactive components and exhibit antioxidant and anti-inflammatory properties, which may be beneficial for arthritis treatment, characterized by chronic inflammation. They are rich in polyunsaturated fatty acids, particularly omega-3, making them beneficial in

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controlling various diseases [8, 9]. Purslane extract has been shown to improve arthritis associated with obesity, likely due to its anti-inflammatory properties [10]. Bakery products fortified with purslane and pumpkin demonstrated high acceptability, in addition to their health benefits [11, 12].

Despite their nutritional richness, little is known about the effects of pumpkin and purslane seeds on bone health. Therefore, the present study aimed to (i) investigate the impact of a high-fat diet on bone and liver histopathology in rats, (ii) evaluate the preventive and therapeutic effects of *Portulaca oleracea* and *Cucurbita moschata* seeds, individually and in combination, on obesity-induced bone defects, and (iii) characterize the nutritional and phytochemical composition of the seeds. Additionally, bakery products fortified with seed flour were developed and assessed for nutritional and sensory quality.

2. Materials and methods

2.1. Plant materials.

Pumpkin and purslane seeds were obtained from a Haraze spices dealer in Egypt in April 2023. Mrs. Therese Labib, a consultant of plant taxonomy at the Ministry of Agriculture and Director of the Orman Botanical Garden, Giza, Egypt, identified the seeds. The seeds were ground into a fine powder and kept for further studies.

2.2. Preparation of successive extractives

The air-dried powder of the tested seeds (500g each) was separately extracted at room temperature by the maceration method with 70% aqueous methanol till exhaustion and then filtered off. The filtrates were concentrated under reduced pressure to dryness at 55 °C, producing 60 and 45 g dried extract of pumpkin and purslane, respectively. The dried extracts were separately suspended in water and consecutively extracted by partition with petroleum ether (60-80 °C), chloroform, ethyl acetate, and butanol until exhaustion in a separating funnel. In a rotary evaporator, each fraction was separately evaporated and weighed.

2.3. Phytochemical investigation

Each fraction was screened for its phytoconstituents, including carbohydrates, alkaloids, coumarins, saponins, tannins, flavonoids, sterols, and triterpenes using the standard qualitative procedures previously outlined [13].

2.4. HPLC analysis for amino acids

2.4.1. HPLC conditions

An Agilent 1260 series was used for the HPLC analysis. Eclipse Plus C18 column (4.6 mm x 250 mm i.d. µm) was used for the separation. The mobile phase, with a flow rate of 1.5 mL/min, contains buffer (sodium borate and sodium phosphate dibasic), pH 8.2 (A), and ACN: MeOH: H2O 45:45:10 (B). The column was kept at a constant temperature of 40 °C.

2.4.2. Preparation of the sample for HPLC analysis

A mixture of 2.5 mL of HCl, 2.5 mL of water, and 0.1g of the powdered plant was heated at 100°C for 24 hours before being filtered. The filtrate (1 mL) was dried and reconstituted in 0.1 M HCl, to be ready for injection into HPLC [14].

2.5. HPLC analysis of vitamins

The presence of water-soluble vitamins C& B (B1, B2, B3, B6, B9, and B12) and fat-soluble vitamins (vitamins A, E, and D) in the powder of pumpkin and purslane seeds was detected using HPLC analysis. An Agilent 1260 series was used for the analysis of water-soluble vitamins. ZORBAX SB-C8 (4.6 mm x 150 mm i.d., 5 µm) was used for the separation. The mobile phase, with a flow rate of 1 ml/min, consisted of 50 mM Sodium phosphate (pH 2.5)/MeOH (90:10) (A) and Sodium phosphate (pH 2.5)/MeOH (10:90) (B). The injection volume is 5 µL. The multi-wavelength detector was monitored at 270 nm. For fat-soluble vitamins, the column used was Agilent C18 (4.6 mm x 250 mm i.d., 5 µm). The mobile phase, with a flow rate of 1.2 mL/min, was methanol: acetonitrile 65:35. The injection volume was 20 µL for each of the sample solutions. The DAD was adjusted at 325 nm. The fluorescence detector was maintained at 290/330 nm (Excitation/Emission). The column was kept at a constant temperature of 40 °C [15].

2.6. Analysis of mineral contents

Contents of phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, zinc, and copper were evaluated in the crushed seeds. Analyses of metal ion concentrations were done using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) Model Agilent 5100 Synchronous Vertical Dual View (SVDV) Serial No. MY15180008 [16].

2.7. Experimental design

The study plan is shown in Figure 1. This study was carried out following the recommendations of the Institutional Animal Ethical Committee (I.A.E.C) with ethical approval no (cu II F 45 23). At the end of each experiment, all rats were sacrificed, and femur and limb bones were removed for measurement of bone density and mineral content through histopathological analysis.

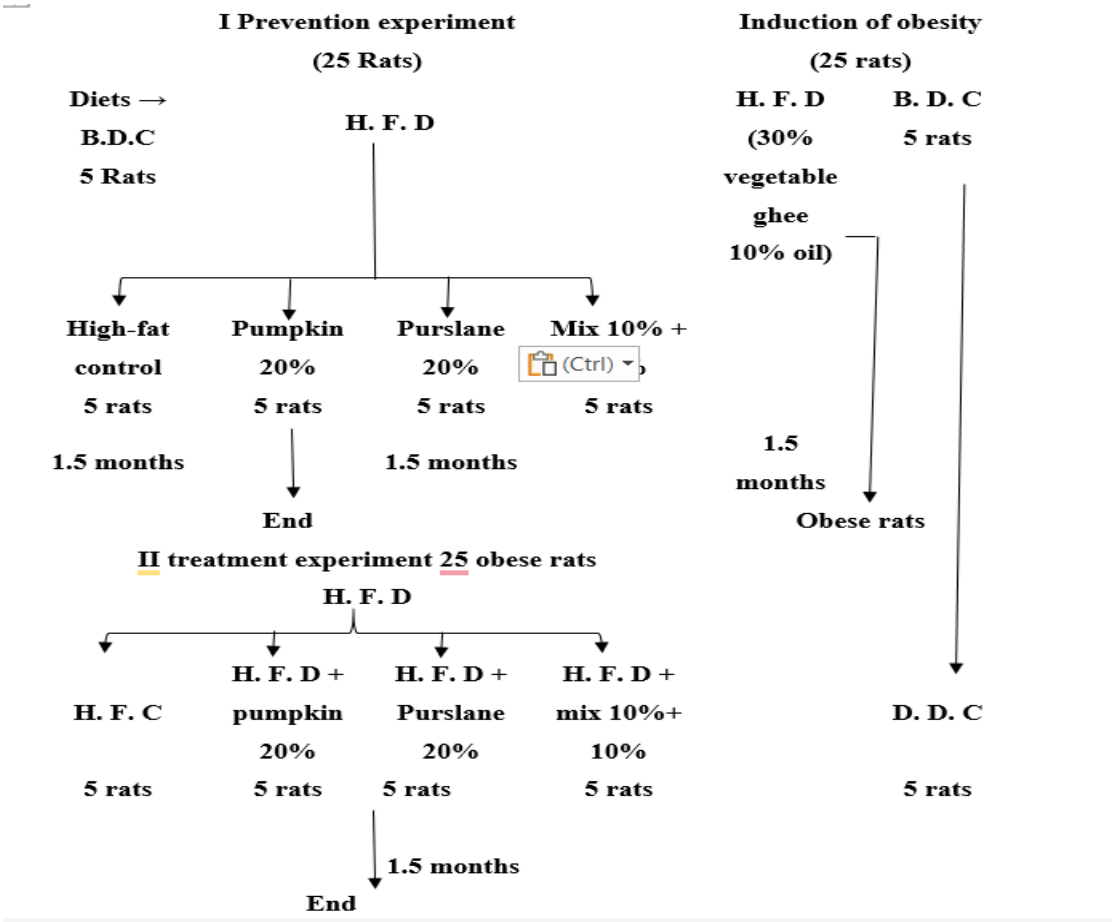

Figure 1: The plan of the experimental study

2.8. Histopathological examination

Liver and femur bone samples were collected, fixed in neutral buffered formalin 10%, and decalcified. All samples were washed, dehydrated, cleared, and embedded in paraffin. The paraffin-embedded blocks were sectioned at 5-micron thickness and stained with Hematoxylin and Eosin, also alizarin red stain was used to evaluate calcium precipitation in femur bone [17] for histopathological examination. Sections were examined by light microscope (Olympus BX50, Japan).

2.9. Histopathological and histochemical lesion scoring

Recorded histopathological lesions in liver and femur bone were scored as, no changes (0), mild (1), moderate (2) and severe (3) changes, the grading was assessed as follows: <30% changes (mild change), <30% – 50% (moderate change), and >50% (severe change) [18, 19]. Alizarin red stain was evaluated and quantified as area% % according to Baraka et al. (2023), [20] by Image J 1.52 p software (Wayne Rasband, National Institutes of Health (U.S.A.)).

2.10. Preparing Bakery Products

Three bakery products (toast, crackers, and cakes) were prepared following the procedures mentioned by Saba (2002), [21] using purslane, pumpkin, and a mix of them (in different percentages) along with other ingredients, as shown in Tables 1-3. The percentage of the ingredients was adjusted to improve the quality of the product and the consistency of the mixture to avoid the dough from spreading and to avoid bitterness.

Table 1: Ingredients of toasts per 100 g in control and fortified (pumpkin, purslane, and a mixture of them)

Group	Oats	Pumpkin	Purslane	Oil	Sesame	yeast	Milk
Pumpkin toast	47	10	-	2.5	12	4.5	24
Purslane toast	47	-	10	2.5	12	4.5	24
Mix toast	47	5	5	2.5	12	4.5	24
control	57	-	-	2.5	12	4.5	24

Table 2: Formulas per 100 g of control and fortified (pumpkin, purslane, and a mixture of them) crackers

Group	Oats	Pumpkin flour	Purslane flour	Honey	Yeast	Butter
Pumpkin crackers	32.5	40	-	12	2.5	13
Purslane	32.5	-	40	12	2.5	13
Mix	32.5	20	20	12	2.5	13
Control	72.5	-	-	12	2.5	13

Table 3: Formulas per 100 g of control and fortified (pumpkin, purslane, and a mixture of them) cakes

Products	Oats	Pumpkin flour	Purslane flour	Baking powder	Vanila	Honey	Egg	Milk	Butter
Pumpkin cake	**26**	**26**	**-**	**2.7**	**1.3**	**13**	**14**	**7**	**10**
Purslane cake	**26**	**-**	**26**	**2.7**	**1.3**	**13**	**14**	**7**	**10**
Mix cake	**26**	**13**	**13**	**2.7**	**1.3**	**13**	**14**	**7**	**10**
Control	**26**	**-**	**-**	**2.7**	**1.3**	**13**	**14**	**7**	**10**

2.11. Chemical analysis

Gross chemical analysis (moisture, protein, fat, ash, and fiber) for each product was conducted according to A 0 A C (2005) [22]. The following equation was used to calculate the amount of carbohydrates:

Carbohydrates=100- (% moisture + protein + fat + ash + fiber).

2.12. Sensory evaluation

Sensory evaluation was conducted on each product to evaluate the flavor, aroma, texture, color, and acceptability using 15 random panelists chosen from the staff and students of the Faculty of Agriculture, Cairo University. All volunteers were informed of their participation, and informed consent was obtained from all participants. The sensory study protocol was reviewed and approved by the institutional ethics committee of Cairo University, with ethical approval no. (cu II F 45 23). The mean value of each sensory property was calculated.

2.13. Statistical analysis

The data obtained from the present study were statistically analyzed using one-way analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$. Statistical analyses were performed using the Web Agricultural Statistics Software Package (WASP). Results are presented as mean ± standard deviation (SD).

3. Results and discussion

3.1. Bakery products

Data concerning sensory evaluation of products (cake, toast, and crackers) fortified with either pumpkin, purslane, or a mix of them, as well as a control, is presented in Table 4. Concerning cake, the sample fortified pumpkin seed flour showed the highest acceptability regarding color, taste, odor, texture, and overall acceptability. It is worth mentioning that no significant difference was found between the pumpkin and control cakes. In contrast, cakes fortified with purslane, or a mixture of purslane and pumpkin flour, showed less acceptability and a statistically significant difference from the control cake.

Table 4: Sensory evaluation of control and fortified cakes, toast, and crackers

Sample	Color	Taste	Odor	Texture	Acceptability
Cakes					
Pumpkin	**8.2±0.41** ^{b}	**8.4±0.38** ^{a}	**9.1±0.81** ^{a}	**8.3±0.59** ^{a}	**8.9±0.91** ^{a}
Purslane	**5.4 ±0.67** ^{d}	**5.1±0.71** ^{b}	**6.1±0.36** ^{b}	**6.9±0.85** ^{b}	**5.6 ±0.65** ^{c}
Mix	**6.4±1.10** ^{c}	**5.9±0.22** ^{b}	**6.7±0.99** ^{b}	**7.1±0.76** ^{b}	**6.7±0.54** ^{b}
Control	**9.5±0.24** ^{a}	**9.5±0.56** ^{a}	**9.6±0.18** ^{a}	**9.4 ±0.44** ^{a}	**9.4±0.77** ^{a}
Toast					
Pumpkin	**5.2±0.56** ^{c}	**5.3±0.79** ^{b}	**5.6±1.03** ^{b}	**5.9±0.61** ^{c}	**5.6±0.93** ^{b}
Purslane	**7.53±0.89** ^{b}	**7.8±0.41** ^{a}	**8.05±0.74** ^{a}	**7.4±0.58** ^{b}	**8.2±0.61** ^{a}
Mix	**5.4±0.72** ^{c}	**5.2±0.82** ^{b}	**4.2±0.92** ^{c}	**4.8±0.38** ^{c}	**4.9±1.10** ^{b}
Control	**8.8±0.69** ^{a}	**8.26±0.54** ^{a}	**8.5±0.80** ^{a}	**8±1.21** ^{a}	**8.4±0.86** ^{a}
Crackers					
Pumpkin	**5.5±1.43** ^{b}	**5.3±0.59** ^{b}	**5.6 ±0.52** ^{b}	**5.2 ±0.29** ^{b}	**5.4 ±1.31** ^{b}
Purslane	**5.1±0.79** ^{b}	**5.0±0.64** ^{b}	**5.2 ±0.77** ^{b}	**5.3 ±0.51** ^{b}	**4.9 ±0.29** ^{b}
Mix	**8.7 ±0.94** ^{a}	**8.1 ±0.90** ^{a}	**8.7 ±1.23** ^{a}	**8.4 ±0.73** ^{a}	**7.7 ±0.84** ^{a}
control	**8.8±0.81** ^{a}	**7.8 ±0.89** ^{a}	**8.7 ±0.93** ^{a}	**8.4 ±0.96** ^{a}	**7.9 ±0.55** ^{a}

Values are presented as mean ±standard deviation. Different letters (a-d) indicate significant differences at $p < 0.05$.

Concerning the sensory evaluation of toast, it could be revealed that toast fortified with purslane seed flour showed the highest acceptability, close to that of the control; no significant difference was noted concerning taste, odor, and overall acceptability between purslane toast and control one. It could also be declared that toast fortified with a mixture of purslane and pumpkin flour had the lowest acceptability, odor, taste, and texture.

Crackers made with a mixture of purslane and pumpkin seed flour showed the highest acceptability, color, taste, odor, and texture. No significant difference was found between the mixed crackers and the control. Purslane, as well as pumpkin seed flour crackers, showed the lowest acceptability, color, taste, odor, and texture, with no significant difference between them.

The differences in acceptability between the mixed-seed formulation and single-seed fortification in different products can be attributed to the combined influence of sensory and functional properties contributed by each seed, with ingredients added. When mixing ingredients used with seeds, their flavors, aromas, colors, and textural components may interact either synergistically or antagonistically. In our study, the seed mixture (pumpkin and purslane) provided a more balanced flavor in crackers only compared to some single-seed fortifications (pumpkin or purslane), which occasionally imparted a stronger or more distinctive taste that reduced acceptability. Some products fortified with a single seed, when mixed with the ingredients, allowed a better overall nutrient and fat composition, which enhanced mouthfeel and appearance, contributing positively to panelists' perception.

Data concerning chemical analysis of the control, as well as pumpkin cakes, purslane toast, and mixed crackers that showed the highest acceptability scores, are presented in Table 5. It could be noticed that the carbohydrate content in the pumpkin and control cakes was 44.53g and 46.3g, respectively. The experiment's cake fat content was less than that of the control (14.35 and 18.5g, respectively), and fiber content was more than 10 times that of the control.

It is worth noting that the experimental cake had a higher ash content (about three times that of the control), which indicates a higher mineral content besides an increase in fiber. Both the experimental and control cakes had almost the same amount of protein.

Concerning the chemical analysis of purslane seed flour toast, it was noted that carbohydrate content was lower in experimental toast compared with that of the control; in contrast, ash content was almost double, and fiber content showed a much higher (more than 13 times) amount in experimental toast. Practically the same amounts of fat and protein were found.

Concerning crackers that contain a mixture of purslane and pumpkin (50%, 50%), higher content of fiber (1.4 times), and ash (2.4 times) was found in the experimental crackers. Almost the same content of carbohydrate, protein, and moisture was found, while fat content was slightly lower (0.87%) than that of the control.

Table 5: Chemical analysis of control and fortified cakes, toast, and crackers on a fresh weight basis (g/100g)

products	Groups	Carbohydrate	Fat	Protein	Fiber	Ash	Moisture
Cake	**Control**	46.3	18.5	7	0.3	1.2	26.8
	Pumpkin cake	44.53	14.35	7.17	3.16	3.34	26.69
Toast	**Control**	53.6	3.5	8.4	0.5	1	33
	Purslane toast	45.26	3.8	8.13	4.82	1.17	31.39
crackers	**Control**	59.04	14.7	8.82	4.7	1.5	7.24
	Mix product	68.84	12.91	8.43	6.64	3.6	7.58

In this concern, Manpreet and Sonika (2018) noticed that cake pumpkin seed flour was higher in protein, fat, fiber, and ash contents [23]. Biatek et al. (2015) found that muffins with pumpkin seed flour had high nutritional value [24]. Substitution of wheat flour (5 and 10%) by pumpkin seed flour in bread increased markedly mineral content [12]. Ahmed et al. (2023) produced biscuits and crackers fortified with purslane and noted an increase in nutritional value, total phenols, flavonoids, antioxidants, and minerals compared with the control [11]. The same results were noted by Shanshan and El Bushaty (2020) [25]**.** Delvarianzadah et al. (2020) found that bread enriched with purslane powder (5 and 10%) showed acceptability in sensory properties, while bread with 15% showed less acceptability, which is parallel with our findings concerning the acceptability of toast fortified with purslane seed flour [26].

Manpreet and Sonika (2018) supplemented cake with pumpkin seed flour (raw and roasted) and mentioned that it showed high acceptability [23]. In this regard, Rodrigues et al. (2018) noticed that the cake that contained 50% pumpkin seed flour and 50% wheat flour was close to the control one [27]. Biatek et al. (2015) also revealed that muffins supplemented with 33% pumpkin seed flour were evaluated by more than 71% as tasty and very tasty [24]. Ahmed et al. (2023) noted that sensory evaluation of bakery products supplemented with purslane (5 and 10%) was accepted [11]. In contrast to that, Shanshan and El Bushuty (2020) found that supplementation of (baton salee, croissants, pizza) with 14% purslane flour was not accepted [25].

3.2. Histopathological findings

3.2.1. Liver

Concerning basal diet control (GI), this group revealed the normal histological structure of liver (Fig. 2a). GII high-fat diet group (after 1.5 months) revealed severe diffuse vacuolar degeneration of hepatocytes (Figs 2 b & c). Amelioration effect was shown in both purslane groups: prevention (GIII) and treatment (GVI), as moderate hepatocellular vacuolar degeneration of liver (Figs. 2d & g) was seen. Both pumpkin feeding group (GIV) and group fed a mixture of pumpkin and purslane seeds (G V) caused the highest prevention effect as well as treated effect (pumpkin G VII and mix G VIII) as improvement in the aforementioned lesions was revealed and hepatocellular degeneration was mild or even hepatocytes were normal (figs. 2 e, f, h &i).

3.2.2. Femur bone

Basal diet control revealed normal histological structure of diaphyseal compact bone and epiphyseal sponge bone (Figs. 3a & b). High-fat diet control groups (after 1.5 months and 3 months) revealed thinning in multiple areas of compact bone (Figs. 3c & e), epiphysis showed thinning in bone trabeculae and loose interconnectivity of trabeculae with widening of inter-trabecular space (Fig. 3d & f). Femur bone of purslane prevention GIII revealed few areas of compact bone thinning (Fig. 3 g), epiphysis showed increased thickness of bone trabeculae and decreased intertrabecular space (Fig. 3 h). It is worth mentioning that in pumpkin-fed groups in both prevention and treatment (GIV & GVI), mix feeding groups (GV &GVIII) as well as the purslane treatment group (GVI), diaphysis and epiphysis showed nearly normal bone shaft with mild or even no bony defects, epiphysis showed nearly normal trabecular thickness, bone interconnectivity with narrow intertrabecular space (Figs. 4a-j).

3.2.3. Histopathological lesion score

Recorded lesions in the liver and femur bone were scored according to their severity (Table 6). Basal control GI showed no histopathological alterations in liver and femur bone (score=0), while high fat control GII (1.5 months and 3 months) showed severe lesions (score=3). Purslane prevention GIII and treatment GVI groups showed mild to moderate alterations in liver and femur bone (score 1 and 2). In the pumpkin prevention GIV and treatment GVII groups, as well as mix groups in prevention GV and treatment GVIII groups, the lesion score revealed observable improvement as scores 0 and 1.

3.2.4. Histochemical findings

Figures 5& 6 showed alizarin red-stained femur bone tissue sections of experimental groups, and the percentage % of alizarin red stain to assess the femur bone mineralization. GI showed uniform bone mineralization of both diaphysis and epiphysis (figs. 5a & b). GII (1.5 months and 3 months) showed small areas of alizarin red stain uptake with weak mineralization (Figs.5 c-f). GIII showed a significant increase in alizarin red stain uptake with larger areas of mineralization (Figs.5 g & h). Also, all experiment groups GIV, GV, GVI, GVII, and GVIII revealed increased alizarin red stain uptake with significantly increased alizarin red area% % and femur bone mineralization (Figs. 5 I & j and Fig. 6).

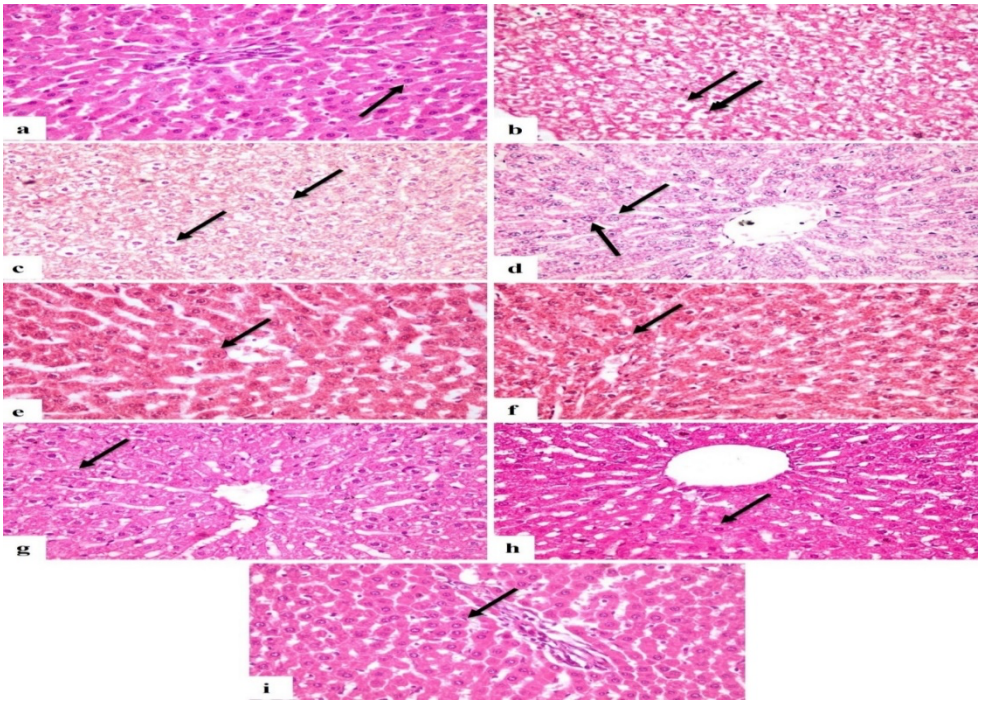

Figure 2: Photomicrograph, rat liver (H& E-stained sections). (a) GI showing normal histological structure of hepatocytes (arrow). (b) GII (1.5 m) showing severe vacuolar degeneration of hepatocytes (arrows). (c) GII (3 m) showing severe diffuse hepatocellular vacuolar degeneration (arrows). (d) GIII shows moderate hepatocellular vacuolar degeneration (arrows). (e) GIV and showing nearly normal hepatocytes (arrow). (f) & (g) GVI & GV showing mild vacuolar degeneration of hepatocytes (arrow). (h) & (i) GVII & GVIII showing normal hepatocytes (arrow) (X200). Keys: GI = basal diet control group. GII = high-fat diet control group, GIII= Purslane

prevention group, GIV = Pumpkin prevention group, GV= Mix prevention group, GVI= Purslane treatment group, GVII = pumpkin treatment group, GVIII = Mix treatment group

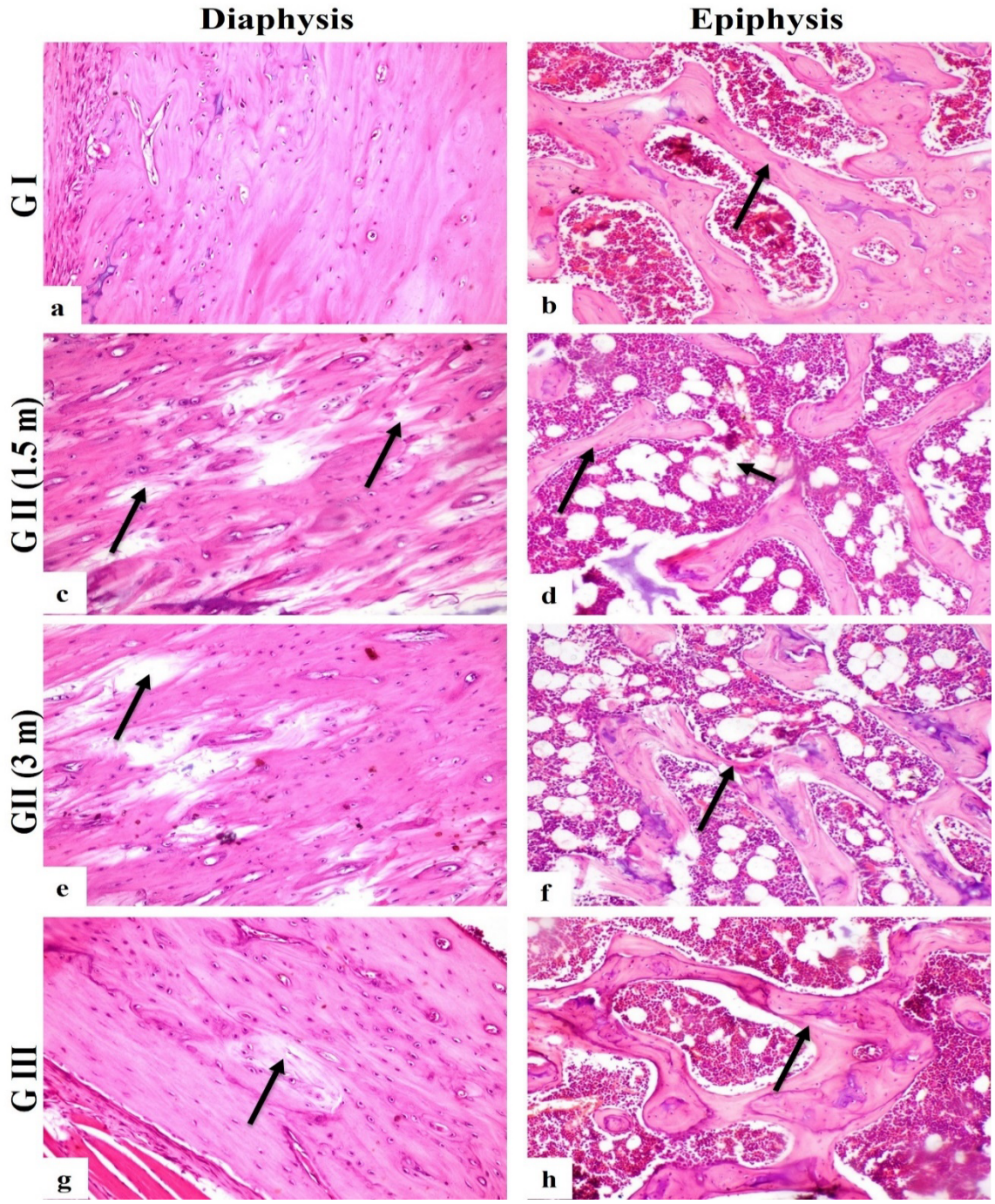

Figure 3: Photomicrograph, rat femur (H& E-stained sections). (a) GI showing normal histological structure of diaphyseal compact bone (b). GI showing normal histological structure of epiphyseal sponge bone (arrow). (c) GII (1.5 m) shows thinning of multiple areas of compact bone (arrows) (d) GII (1.5 m) epiphysis showing thinning in bone trabeculae (long arrow), widening of intertrabecular space (short arrow). (e) GII (3 m) compact bone showing multiple areas of thinning (arrow). (f) GII (3 m) sponge bone showing thinning in bone trabeculae (arrow). (g) GIII diaphysis showing small areas of bone thinning (arrow). (h) GIII epiphysis showing increased trabecular thickness (X100). Keys: GI = basal diet control group. GII = high-fat diet control group, GIII= Purslane prevention group, GIV = Pumpkin prevention group, GV= Mix prevention group, GVI= Purslane treatment group, GVII = pumpkin treatment group, GVIII = Mix treatment group

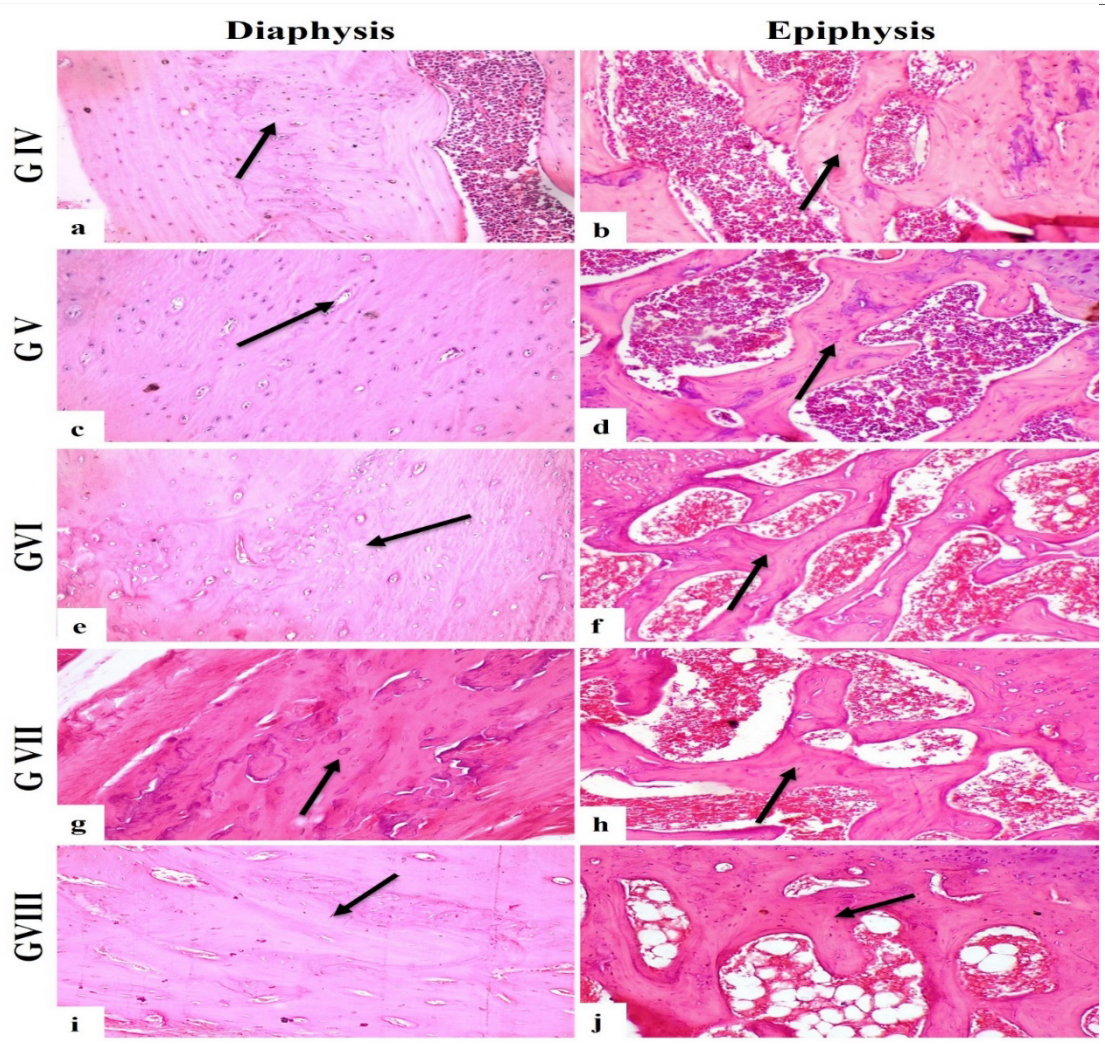

Figure 4: Photomicrograph, rat femur (H& E-stained sections). (a) GIV showing normal bone shaft (arrow). (b) GIV epiphysis showing normal bone trabeculae (arrow) (c) – (j) GIV, GV, GVI, GVII, and GVIII diaphysis and epiphysis showing nearly normal compact bone with few bony defects with increased trabecular thickness, interconnectivity, and decreased intertrabecular space (arrow). (X100). Keys: GI = basal diet control group. GII = high-fat diet control group, GIII= Purslane prevention group, GIV = Pumpkin prevention group, GV= Mix prevention group, GVI= Purslane treatment group, GVII = pumpkin treatment group, GVIII = Mix treatment group

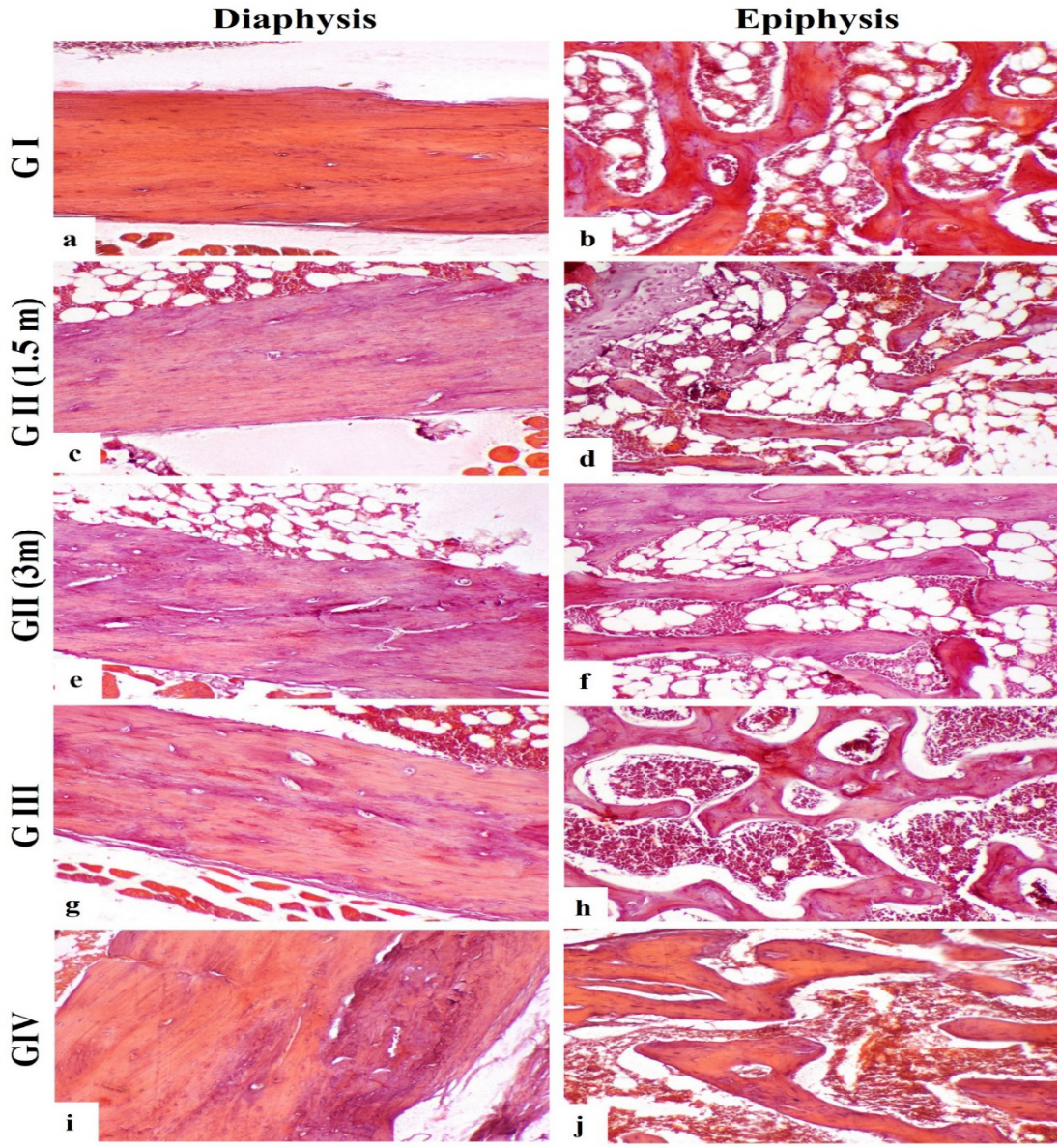

Figure 5: Photomicrograph, rat femur (Alizarin red stained sections X100). (a) & (b) GI showing uniform mineralization of diaphysis and epiphysis. (c-f) GII showing small areas of alizarin red stain. (g) & (h) GIII showing large areas of alizarin red uptake in diaphysis and epiphysis. (i) & (j) GIV showing increased stain uptake. (X100). Keys: GI = basal diet control group. GII = high-fat diet control group, GIII= Purslane prevention group, GIV = Pumpkin prevention group, GV= Mix prevention group, GVI= Purslane treatment group, GVII = pumpkin treatment group, GVIII = Mix treatment group

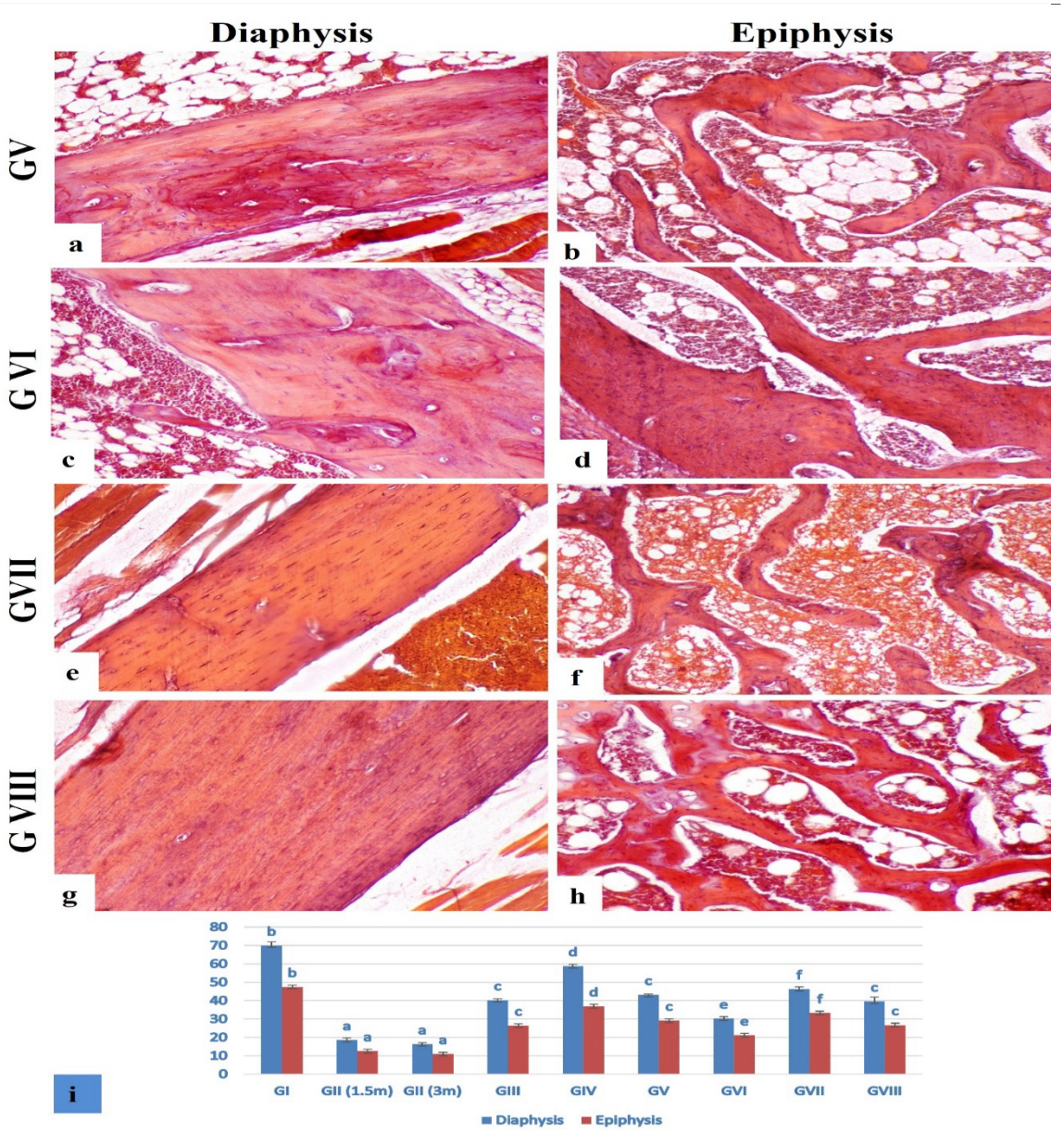

Figure 6: Photomicrograph, rat femur (Alizarin red stained sections X100). (a)- (h) GV, GVI, GVII, and GVIII diaphysis and epiphysis showing increased intensity of alizarin red stain uptake with variable degrees. (i) area% % of alizarin red stain of femur bone (data was expressed as mean ±SE, different letters indicating significant differences at $p < 0.05$). Keys: GI = basal diet control group. GII = high-fat diet control group, GIII= Purslane prevention group, GIV = Pumpkin prevention group, GV= Mix prevention group, GVI= Purslane treatment group, GVII = pumpkin treatment group, GVIII = Mix treatment group

Table 6: Lesion score of histopathological alterations in the liver and femur bone of different treated groups

Lesions	GI	**GII (1.5 & 3 m)**	**GIII**	**GIV**	**GV**	**GVI**	**GVII**	**GVIII**
LIVER: **Vacuolar degeneration of hepatocytes**	0	3	2	0	1	2	0	1
Femur bone:								
Thinning of compact bone	0	3	2	1	2	2	1	2
Thinning of epiphyseal bone trabeculae	0	3	2	1	1	1	1	1
Loose trabecular inter-connectivity	0	2	1	0	1	1	1	1
Widening of the inter-trabecular space	0	3	1	1	1	2	1	1

The score system was designed as follows: score 0 = absence of the lesion in all rats of the group (n=5), score 1= (<30%), score 2= (<30% – 50%), score 3= (>50%). GI = basal diet control group. GII = high-fat diet control group, GIII= Purslane prevention group, GIV = Pumpkin prevention group, GV= Mix prevention group, GVI= Purslane treatment group, GVII = pumpkin treatment group, GVIII = Mix treatment group

3.3. Phytochemical screening and the yield of different extracts of pumpkin and purslane seeds

Table 7 represents the results of a phytochemical screening of different fractions of the tested seeds. It showed that carbohydrates and saponins were mainly found in 70% methanol fractions of the tested seeds. Alkaloids were detected in the ethyl acetate fraction and the 70 % methanol extract of pumpkin seeds, while they were found in the chloroform fraction and the 70 % methanol extract of purslane seeds.

Furthermore, the results revealed the presence of coumarins in the chloroform fractions and 70 % methanol extracts of the tested seeds; coumarins were also detected in the ethyl acetate fraction of pumpkin seeds. Triterpenes and/or sterols were detected in all fractions of the tested seeds. Flavonoids were detected in the butanol fractions and 70% methanol extracts of the tested seeds; they were also detected in the chloroform and ethyl acetate fractions of pumpkin and purslane, respectively. In addition, tannins were found in the 70% methanol extracts of the tested seeds. They were also detected in the butanol fraction of purslane seeds. Furthermore, the yield of different fractions was detected, which indicated that the yield of petroleum ether, chloroform, ethyl acetate, butanol fractions, and 70 % methanol extract (12.3 & 9.6g), (7.5 & 4.6 g), (9.4 & 6.3 g), (11.7 & 8.3 g), and (14.5 &10.5 g) in pumpkin and purslane, respectively. The current findings agree with the reported ones, which revealed that pumpkin and purslane seeds are rich in a diversity of bioactive phytoconstituents [28, 29].

Table 7: Results of phytochemical screening of successive extractives of pumpkin and purslane seeds

Fractions	Weight (g) 1 2	Tannins 1 2	Flavonoid 1 2	Sterols/ triterpenes 1 2	Saponins 1 2	Coumarins 1 2	Alkaloids 1 2	Carbohydrate 1 2
Pet. Ether (60-80 °C)	12.3 9.6	- -	- -	+ +	-	- -	-	-
Chloroform	7.5 4.6	- -	+ -	+ +	-	+ +	- +	-
Ethyl Acetate	9.4 6.3	- -	- +	+ +	-	+ -	+	-
Butanol	11.7 8.3	- +	+ +	+ +	-	-	-	-
70%Methanol extract	14.5 10. 5	+ +	+ +	+ +	+ +	+ +	+ +	+ +

1: pumpkin; 2: purslane; + :The presence of the constituents; -:The absence of the constituents

3.4. HPLC analysis of amino acids

The HPLC chromatograms of amino acids in purslane and pumpkin seeds are represented in Figures 7 and 8, respectively. The identified amino acids in both plants are found in Table 8. Sixteen amino acids were identified in each plant with different concentrations. The results showed that the concentration of non-essential amino acids in purslane (70.115 mg/g) and pumpkin (164.51 mg/g) was higher than that of essential amino acids (27.059 and 60.891 mg/g, respectively). Pumpkin seeds contain a higher concentration of amino acids than purslane. Glutamic acid was the major amino acid in purslane and pumpkin seeds (18.979 and 46.819 mg/g, respectively). Amino acids are essential building blocks for proteins and metabolic intermediates. The human body's physiological processes depend equally on the dietary supply of sufficient amounts and high-quality essential amino acids [30]**.** The current findings agree with the reported ones, which have reported that pumpkin seeds are rich in a high amount of amino acids [30, 31]**.** Our results revealed that glutamic acid, arginine, and aspartic acid were the predominant non-essential amino acids in pumpkin and purslane seeds, which agrees with that mentioned by Rayan et al. (2023) [32]**.** Purslane and pumpkin seed had ratios of 27% total essential amino acids to total amino acids, which is higher than the recommended amount for a protein-rich diet for adults (11%) and children (26%), respectively [32, 33]**.** A previous study reported that essential amino acids could decrease osteoclast activity, increase bone mass, osteoblast proliferation, and differentiation [34]**.** Furthermore, a study revealed that L-Arginine exhibited a powerful effect on fracture healing [35]**.**

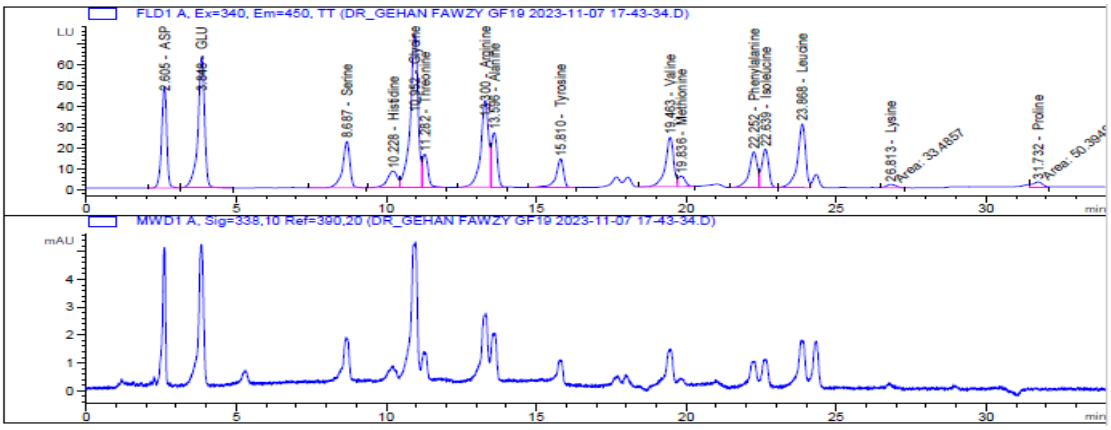

Figure 7: HPLC analysis of amino acids in *Portulaca oleracea* (purslane) seeds

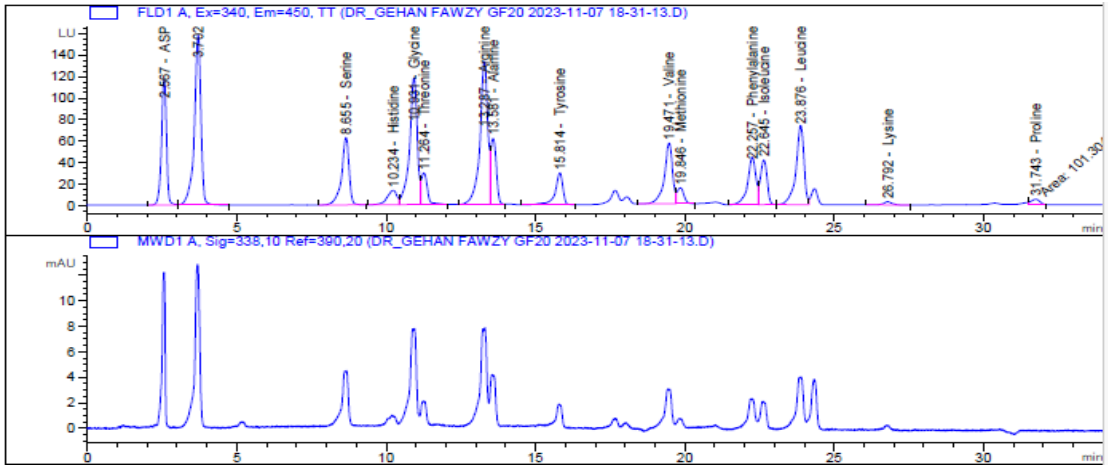

Figure 8: HPLC analysis of amino acids in *Cucurbita moschata* (pumpkin) seeds

Table 8: HPLC analysis of amino acids in purslane and pumpkin seeds

Amino acid		**Purslane**	**Pumpkin**
		Concentration (mg/g)	
Essential amino acid	- Lysine	4.812	8.127
	- Leucine	**6.304**	**15.237**
	- Isoleucine	3.730	8.407
	- Phenylalanine	4.225	**10.931**
	- Methionine	1.027	3.026
	- Valine	3.707	8.905
	- Threonine	3.254	6.258
Total essential amino acid		27.059	**60.891**
Non-essential amino acid	- Tyrosine	4.403	9.557
	- Alanine	3.929	8.750
	- Arginine	**10.021**	**32.263**
	- Proline	4.552	9.150
	- Glycine	8.637	13.808
	- Histidine	5.871	9.572
	- Serine	4.287	12.345
	- Glutamic acid	**18.979**	**46.819**
	- Aspartic acid	**9.436**	**22.246**
Total non-essential amino acid		**70.115**	**164.51**
Total amino acids		**97.174 mg/g**	**225.491 mg/g**
Ratio of total essential amino acids to total amino acids		**27.8**	**27.0**

3.5. HPLC analysis of vitamins

The HPLC chromatograms of the water-soluble vitamins in purslane and pumpkin seeds are illustrated in Figures 9 and 10, respectively. The identified water-soluble vitamins in each plant are listed in Table 9. The results revealed that purslane seeds contained vitamins B1 (62.57 µg/g), B2 (8.08 µg/g), B3(330.43 µg/g), and B9 (39.58 µg/g). It was found that vitamin B3 was

the predominant vitamin in purslane seeds. On the other side, pumpkin seeds contained B1 (90.71 µg/g), B2 (1.84 µg/g), B6 (84.99 µg/g), and B12 (4.76 µg/g). Vitamins B1 and B6 were the predominant vitamins in pumpkin seeds. Regarding Vitamin C, purslane seeds contained a higher content (237.70 µg/g) than that of pumpkin seeds (103.79 µg/g). It was reported that vitamin C exhibits an antioxidant effect and plays a crucial role in collagen formation [36]**.** The HPLC chromatograms of fat-soluble vitamins in purslane and pumpkin seeds are illustrated in Figures 11 and 12, respectively. The identified fat-soluble vitamins in each plant are listed in Table 9. The results showed that vitamins A and D were detected in both plants at different concentrations. Vitamin E was detected only in pumpkin seeds (2.423 µg/g). Pumpkin seeds contained a higher content of vitamin A (1.172 µg/g) than that of purslane seeds (0.250 µg/g). Vitamin A stimulates skeletal growth, which is necessary for bone remodeling [36]**.** Previous studies revealed that purslane and pumpkin seeds contain various vitamins that play a crucial role in human health [37-39]**.**

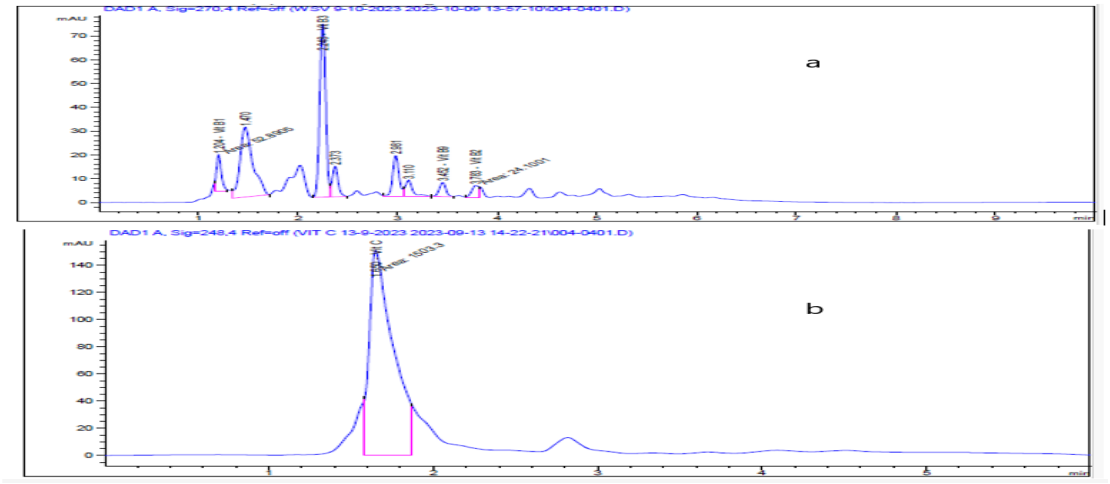

Figure 9: HPLC chromatogram of water-soluble vitamins; a: vitamins B, b: Vitamin C in purslane seeds

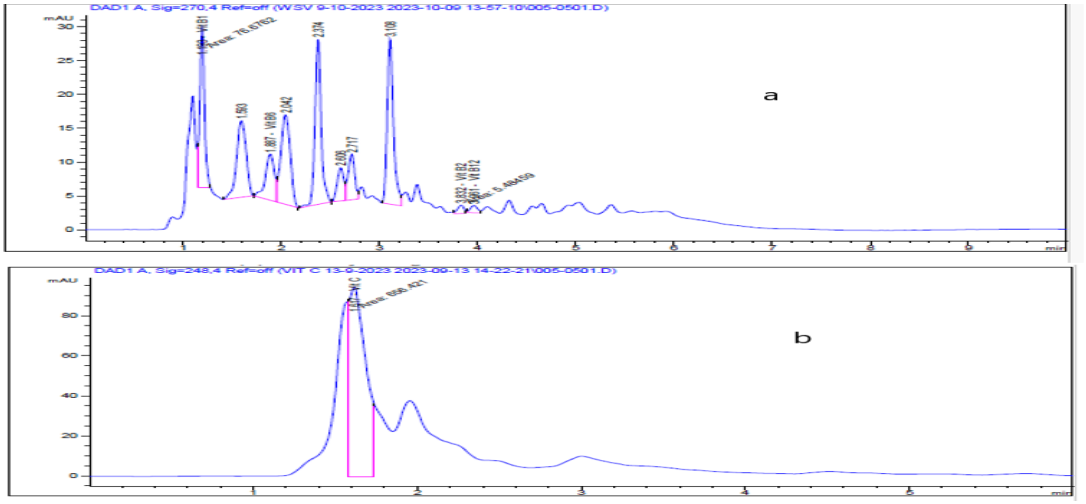

Figure 10: HPLC chromatogram of water-soluble vitamins; a: vitamins B, b: Vitamin C in pumpkin seeds

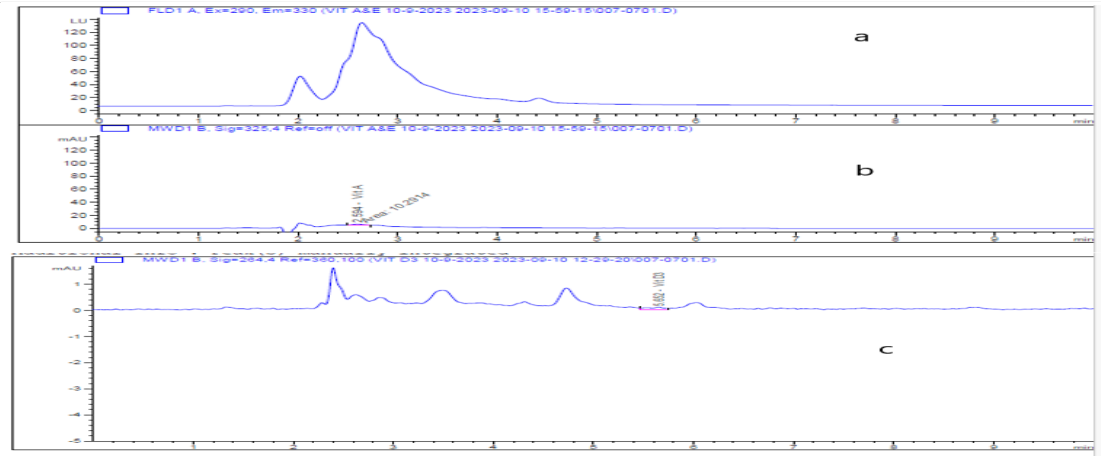

Figure 11: HPLC chromatogram of fat-soluble vitamins in purslane seeds: a: vitamin E; b: vitamin A; c: vitamin D

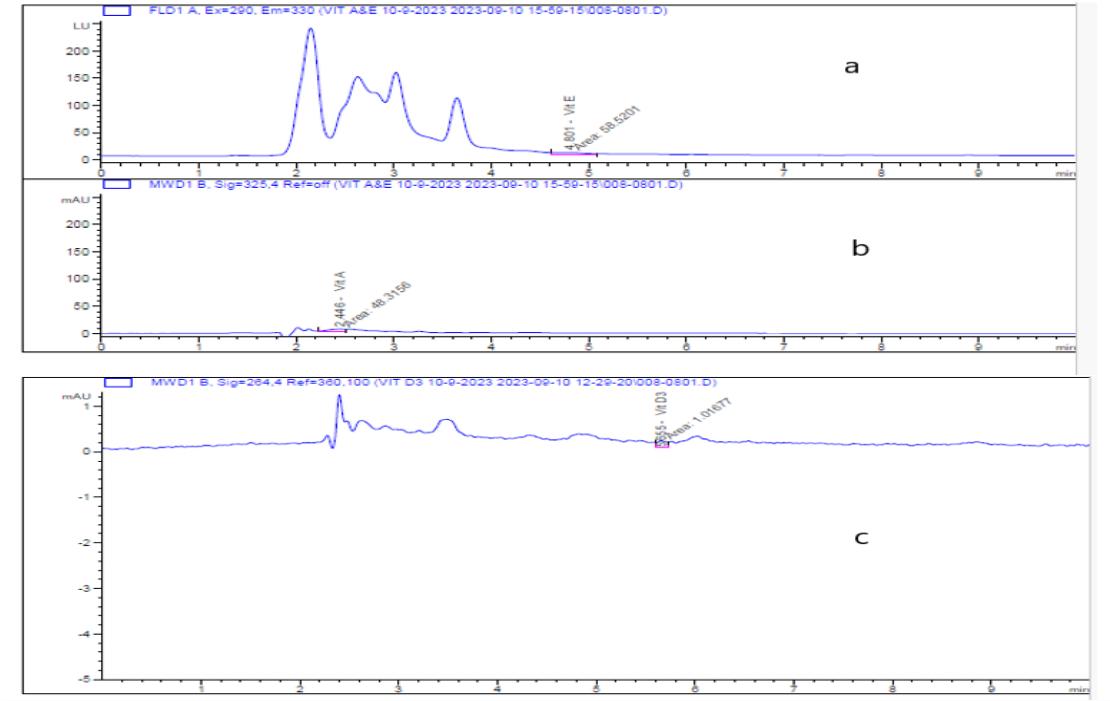

Figure 12: HPLC chromatogram of fat-soluble vitamins in pumpkin seeds: a: vitamin E; b: vitamin A; c: vitamin D

Table 9: HPLC analysis of vitamins in purslane and pumpkin seeds

Vitamins	Purslane seeds	Pumpkin seeds
	Concentration (µg/g)	
Water-soluble vitamins		
Vit. B12 (Cobalamin)	0.00	4.76
Vit. B9 (Cyanocobalamin)	39.58	0.00
Vit. B6 (Pyridoxine)	0.00	**84.99**
Vit. B2 (Riboflavin)	8.08	1.84
Vit. B1 (Thiamin)	62.57	**90.71**
Vit. B3 (niacin)	**330.43**	0.00
Vit. C	**237.70**	**103.79**
Fat-soluble vitamins		
Vit. D	0.117	0.095
Vit. E	0.000	2.423
Vit. A	0.250	1.172

3.6. Analysis of mineral contents

Table 10 represents the mineral content of purslane and pumpkin seeds. Each plant contains macro elements (sodium, magnesium, calcium, potassium, and phosphorus) and micro elements (copper, zinc, manganese, and iron) in various concentrations. The current findings revealed that purslane and pumpkin seeds are rich in many vital minerals, which are important for the proper functioning of our muscles [31]. The concentration of both total macro elements and total microelements in purslane (11850 and 150 mg/kg, respectively) is higher than that of pumpkin seeds (9600 and 100 mg/kg, respectively). The current results revealed that purslane and pumpkin seeds are rich in potassium (5000 and 3675mg/kg, respectively), which agrees with the previous studies, which reported that purslane and pumpkin seeds were rich in potassium, which plays an important role in muscle contraction [31, 37, 39]. In addition, magnesium was found in purslane and pumpkin seeds with high concentrations (1750 and 1250 mg/kg, respectively). Magnesium is an important element for the structuring of the skeletal system [31, 37]. Phosphorus was detected in each plant with the same concentration, constituting 3750 mg/kg. All the body's cells require phosphorus for healthy bodily function [31]**.** Purslane seeds contain a higher content of calcium (1100 mg/kg) than that of pumpkin seeds (375mg/kg). The high concentration of calcium in purslane was previously reported by Kumar et al. (2021) [39]. Purslane and pumpkin seeds contain a high concentration of iron (77.5 and 47.5 mg/kg, respectively). Iron is essential for the transportation of oxygen by myoglobin in muscles and haemoglobin in blood [31]**.**

Table 10: Contents of the macro and micro elements in purslane and pumpkin seeds

Content	Purslane seeds	Pumpkin seeds
Macro elements	**Concentration (mg/kg)**	
Na	250	550
Mg	1750	1250
Ca	**1100**	375
K	**5000**	3675
P	3750	3750
Total	**11850**	**9600**
Microelements		
Cu	5.75	3.5
Zn	45	40
Mn	31.75	9
Fe	**77.5**	**47.5**
Total	**160**	**100**

The current bioactivities of the tested seeds may be due to the synergistic effect of their nutritive content and phytoconstituents. Glutamic acid, the major amino acid in purslane and pumpkin seeds, plays a crucial role in energy metabolism and serves as a precursor for neurotransmitters involved in appetite regulation, which may contribute to reduced food intake and fat accumulation [40]. Additionally, B vitamins (particularly B1, B3, and B6) act as essential cofactors in carbohydrate and lipid metabolism, thereby improving energy utilization and limiting lipid deposition [41]. The current study demonstrates that purslane and pumpkin, rich in essential nutrients needed for bone health (essential and non-essential amino acids, minerals like Ca, P & Mg, as well as Vit D), can also be effective treatments for bone damage caused by a high-fat diet and obesity. Moreover, they can be effectively incorporated into products with acceptable sensory properties. We are proud that effective seeds for bone health (pumpkin/purslane and mix) can be successfully incorporated into palatable food products (cake/crackers) for human consumption. This integration represents a significant advancement in developing therapy that balances efficacy with consumer acceptability, thereby enhancing potential adherence and practical application.

4. Conclusion

Bone health is a crucial factor in determining overall human vitality and activity. It is essential to focus on maintaining bone health by ensuring a balanced intake of essential nutrients from natural sources such as pumpkin and purslane seeds. Reducing the consumption of high-fat foods and obesity is vital, as they negatively impact bone health. Our findings demonstrate the effectiveness of pumpkin and purslane seeds in managing the negative impacts on bones that may arise from obesity or high-fat diets. Moreover, pumpkin and purslane seeds are abundant in vitamins, minerals, and amino acids. It is also important to encourage industries to utilize these beneficial natural ingredients in their products, such as bakery items, to create accessible and healthy options for consumers, thereby promoting overall well-being and bone health. Clinical studies are needed to confirm the effects of purslane and pumpkin seed supplementation on bone density and overall skeletal health.

Author contributions

G. Raoof, R. Mourad, and H. Nagy suggested and designed the study; G. Raoof performed all the phytochemical studies, analyzed and characterized the data, and wrote and edited the manuscript. R. Mourad and H. Nagy performed the biological study, prepared the products, and related sensory and chemical analysis; analyzed and characterized the data, and wrote the manuscript. R. Ibrahim performed the histopathological part, analyzed and characterized the data, and wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data supporting this article have been included as part of the Supplementary Information.

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