#### **INTRODUTION:**

Osteoporosis represents one of the major health and socioeconomic problems of the modern society (Prelević **.1991**). Osteoporosis is a skeletal condition characterized by decreased density (mass/volume) of normally mineralized bone. The reduced bone density leads to decreased mechanical strength, thus making the skeleton more likely to fracture. Postmenopausal osteoporosis (Type I) and agerelated osteoporosis (Type II) are the most common primary forms of bone loss seen in clinical practice. Secondary causes of osteoporosis include hypercortisolism, hyperthvroidism, hyperparathyroidism, alcohol abuse, and immobilization. In the development of osteoporosis, there is often a long latent period before the appearance of the main clinical manifestation, pathologic fractures. The earliest symptom of osteoporosis is often an episode of acute back pain caused by a pathologic vertebral compression fracture, or an episode of groin or thigh pain caused by a pathologic hip fracture. (Glaser and Kaplan, 1997)

Prunes are dried plums and consumed as food or medicine. Prunus domestica, Prunus salicina and Prunus americana are the important sources of prunes. Prunes are highly reputed in folk medical practices for nutritive, laxative and digestive properties and used for treatment of hypertension, diabetes, jaundice and fever. The recent showed that it has antioxidant. studies anticancer. antihyperglycemic, anti-hyperlipidemic, antihypertensive, anti-osteoporosis, laxative and hepatoprotective activities. Prunes contain dietary fibers, carbohydrates, amino acids, vitamins, minerals and antioxidant polyphenolic phytochemicals.(Qaiser and Naveed, 2011)

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In the recent years many studies on anthocyanins have revealed their strong antioxidant activity and their possible use as chemotherapeutics. The finding that sour cherries (*Prunus cerasus* L) (also called tart cherries) contain high levels of anthocyanins that possess strong antioxidant and anti-inflammatory properties has attracted much attention to this species.(**Federica et al, 2004**)

Anthocyanins are particularly high in cherries and highly concentrated in he skin of the fruit, accumulating during the ripening process. Cherries consist of more than 100 different species, of which the "sweet" and "sour" (tart)cherry species are perhaps the most recognized. Sweet and tart cherries are agood source of many vitamins, minerals, and phytochemicals, but tart cherries contain considerably more total phenolics, which has been partially attributed to the higher content of anthocyanins in tart cherries. One of the most frequently encountered anthocyanin in cherries is cyanidin-3-glucoside, and the quantity of anthocyanins in cherries produced by various cultivars has been measured at 30-79 mg of cvanidin-3glucoside equivalents(CGE)/100g in sweet cherries and 45-109 mg CGE/100g in tart cherries . The scientific designation for tart cherries is Prunus cerasus L., and although they are vitamin and nutrient rich, they are not calorically dense .( Katie ,2011)

**Tinna et al., (2009)** Consumption of foods high in polyphenols, particularly anthocyanins, have been associated with improved health, but the mechanisms contributing to these salutary effects remain to be fully established. consumption of tart cherry juice improves antioxidant defenses in vivo in older adults by an increased capacity to constrain an oxidative challenge and reduced oxidative damage to nucleic acids. Anthocyanins are a

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group of natural occurring pigments responsible for the redblue color of many fruits and vegetables. Anthocyanins are of interest for two reasons because they cannot only be used in the technological field as natural colorants but also have important implications in the field of human health. Numerous studies indicate the potential effect that this family of flavonoids may have in reducing the incidence of cardiovascular disease, cancer, hyperlipidemias and other chronic diseases through the intake of anthocyanin-rich foods. (Sonia et al, 2008).

The present work was conducted to study the effect of dried cherries and plumes on rats suffering from osteoporosis.

#### **MATERIALS AND METHODS:**

#### Materials:

- Casein, vitamins, minerals, cellulose and choline chloride were obtained from El Gomhorya Company, Cairo, Egypt.
- Starch and soybean oil were obtained from local market, Cairo, Egypt.
- Cherries and plums were obtained from local market, Cairo, Egypt.
- Normal male albino rats (48) of Sprague Dawley strain were obtained from Helwan farm to experiment animals, Helwan, Egypt.
- Hostacortin or glucocorticoid (prednisone a acetate 5mg) were obtained from a local pharmacy, cairo , Egypt.

#### **Methods:**

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# **Dehydration and chemical analysis of cherries and** plums.

- Dehydration of fresh cherries and plums by using solar energy for 24 at 50  $^{\circ}\mathrm{C}$  .
- The active components (phenolic and flavonoid), chemical composition and mineral of dried cherries and plums were determined.

#### **Biological Investigation:**

Male albino rats Sprague Dawley strain (48 rats) weighing  $(200\pm 10g)$  were housed in individual stainless steel cages under hygienic conditions and fed on basal diet for one week for adaptation at adlibitum in the animal house of faculty of home economics , Helwan University. The basal diet preliminary experiment consist of 14% casein (protein >80%) , soy oil 4%, cellulose 5%, vitamins mixture 1% , salt mixture3.5%, choline chloride 0.25% and the reminder is corn starch (**Reeves et al , 1993**) and the vitamin mixture was prepared according to (A.O.A.C. 1975).

After adaptation period, the rats were divided into two main groups as follows:

The first main group (6 rats): This group was fed on basal diet as a negative control group.

The second main group (42 rats) was fed on basal diet containing 100mg of prednisone /kg diet to induced sever bone loss for 2 weeks according to (li et al., 2003), after the induction with prednisone blood samples were talked from rats (negative and positive control groups) to analysis minerals in the serum (calcium, phosphor and zinc) and using x-ray at femur bones to make sure the induction of osteoporosis.

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This group was divided into 7 subgroups (6 rats each) as following:

Subgroup 1 : fed on basal diet as a control positive group.

**Subgroup 2 and 3** : fed on diets containing (3 and 6% dried cherries ).

**Subgroup 4 and 5 :** fed on diet containing (3 and 6% dried plums ).

**Subgroup 6 and 7 :** fed on diet containing 3 and 6% mix from dried cherries and plums(1:1).

Weights of rats and food consumed were recorded during the experimental period. At the end of the experimental period (4 weeks), rats were fasted over night before sacrificing blood samples collected from each rats and centrifuged at 3000 r.p.m to separate the serum was carefully separated and transferred into dry clean Ebendrof tubes and kept frozen at -20c till analysis.

Femur bones were removed and washed with cold saline and dried between two filter papers, then weighed and kept in formalin station (10%) according to (**Drury and Wallington , 1980**).

#### **Biological evaluation:**

Biological evaluation of the different tested diets was carried by determination of feed intake (FI), body weight gain % (BWG %) and organs weight / body weight% according to Chapman *et al.*, (1959) using the following equation:

Biochemical analysis of serum : Serum glucose was determined according to the method described by *Trinder*, (1959, uric acid by *Fossati et al.*, (1980), urea nitrogen by

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**Patton and Crouch, (1977),** creatinine by **Bohmer, (1971),** AST and ALT activities by **Henry (1974),** alkaline Phosphates by **Belfield and Goldberg(1971),** calcium by (**Baginski, 1973).** phosphorus by (Yee, 1968), sodium and potassium were measured according to the colorimetric method of **Henry** *et al.* (1974) and serum zinc by (**Makino et al., 1982).** 

Bone mineral density (BMD) and bone mineral concentration (BMC) were measured in femur bone of rats after treatment by using Dual energy X-ray absorptiometry (DEXA).

#### Histopathological Examination of femur bone:

Femur bones were fixed, immediately after separation, cleaning and drying by a filter paper, in neutral buffered formalin. After dehydration in alcohol solutions of increasing concentrations, samples were blocked in paraffin wax. Sections were prepared by a retary microtome, stained and investigated by a light microscope (Carleton, 1979).

#### Statistical analysis:

The statistical analysis were carried out by using SPSS, PC statistical software (verion 8.0SPSS Inc., chieago, USA). The results were expressed as mean  $\pm$  SD. Data were analyzed by one way analysis of variance (ANOVA). The differences between means were tested for significance using least significant difference (LSD) test at (P < 0.05) **Steel and Torri., (1980).** 

#### **Results and Discussion:**

Flavonoids and total phenolic compounds in cherries and plumes:

Effect of dried Cherries and Plumes on rats suffering from osteoporosis

The data presented in Table (1) revealed that, the amount of flavonoids and total phenolic in cherries and plumes were (2.35 and 7.89 mg/g) and (2.95 and 17.82 mg/g), respectively. The results of chemical analysis showed decrease in the amount of flavonoids and total phenolic in cherries as compared to the plumes.

**Table (1): Flavonoids and total phenolic compounds in cherries and plumes** (mg/g).

Compounds	Flavonoids	Total phenolic
Sample	mg/g	
Cherries	2.35	2.95
Plumes	7.89	17.82

flavonoids and total phenolic of plumes were increased by about 235.744% and 504.067%, than that of cherries, respectively.

Plums and prunes are rich source of polyphenolic phytochemicals. Total phenolic contents obtained in the current study was agree with total flavonoids content of different plum cultivars have been reported between 282-922 mg/100 g of fruit (Siddiq, 2006).

**Marinova et al.,(2005)** reported that, sour cherry (429.5mg/ GAE100g.**Valentina et al ., (2008)** reported that, Sweet cherry cultivars of different pomological characteristics and different time of ripening were evaluated seniority. Cultivars were evaluated for their total phenolic content and antioxidant activity. The sum of sugars (glucose, fructose, sucrose and sorbitol) ranged from 125 to 265 g/kg fresh weight (FW).

Total phenolics in sweet and sour cherries per 100 g ranged from 92.1 to 146.8 and from 146.1 to 312.4 mg

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gallic acid equivalents, respectively. (Dao et al, 2005)

Calcium, zinc and phosphorus in cherries and plumes:

The amount of calcium, zinc and phosphorus in cherries and plumes were (1620, 3.00 and 65.88 mg/100g) and (1770, 1.00 and 74.89 mg/100g), respectively. The results in Table (2) showed increase the amount of calcium and phosphorus in the plumes, than that of cherries. While the amount of zinc was three times more in cherries, than that of the plumes.

Table (2): Calcium, and phosphorus contents in cherries and plumes (mg/100g).

Minerals Sample	Calcium	Zinc	Phosphorus
Cherries	1620	3.00	65.88
Plumes	1770	1.00	74.89

The amount of calcium and phosphorus increased in plumes by about 9.259% and 13.676%, than that of the cherries, while the amount of zinc in cherries increased by about 200%, than that of the plumes. **Tomo and Nebojša**, (2012) reported that , The average content of ash in plum fruits reached 0.78%, phosphorus – 0.06%, potassium – 1.45%, calcium and zinc – 19.29  $\mu$ g g–1 of dry matter.

**Vunchi et al ., (2011)** reported that, samples of Vitex doniana fruit (black plum) were collected from Vunchi village in Bida metropolis. The

dried endocarp was washed, ground into powder and analysed for the proximate, vitamins and mineral compositions. The values (%DW) of moisture, ash, crude protein, crude fibre, crude fat and carbohydrate available

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were 16.66, 11.50, 8.24, 0.58, 34.62 and 28.40, respectively. Levels of Vitamin A, B1, B2, B6 and C were 0.27, 18.33, 4.80, 20.45 and 35.58 (mg/100g DW) respectively. Mineral (mg/100g DW) were: potassium (16.5), sodium (10.40), calcium (30.27), and phosphorus (16.50).

**Krośniak et al ., (2010)** reported that cherry juice contained high levels of Ca, reaching 10-fold higher (323 mg/L) levels than other juices (14–77 mg/L). With respect to the remaining elements, K, Na, Fe, Zn, and Mn) Cevdet and Hasan,(1997) reported that calcium, 25.47 mg kg-1.

#### Effect of cherries and plumes dried on feed intake, BWG% and femur bone/body weight% of rats suffering from osteoporosis:

#### Feed intake (g/day/rat):

Results in this Table 3 revealed that, treating rats which were fed on basal diet with prednisone acetate to induce osteoporosis decreased the mean value of feed intake, than that of healthy rats fed on basal diet. The mean value of feed intake in the positive control group decreased by about 13.78%, than that of the negative control group.

The mean values of feed intake in all investigated groups increased, than that of the positive control group. On the other hand, the highest amount of feed intake recorded for the group treated with the combination of 6% (cherries and plumes) "1:1", followed by the groups treated with 6% dried plum and 6% cherries, respectively. While the lowest mean value of feed intake was found in the group treated with the combination of 3% (cherries and plumes) "1:1", followed by the groups which were treated with 3% cherries and 3% plumes, respectively. The mean value of feed intake in osteoporotic group which treated with the high level of

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the combination of cherries and plumes increased by about 10.373%, than that of the control positive group.

#### Body weight gain% (BWG%):

The data in Table (3) revealed that, feeding rats on basal diet with prednisone acetate decreased the mean value  $\pm$  SD of body weight gain% significantly (pl 0.05), than that of healthy rats fed on the same diet, respectively. On the other hand, non-significant changes in body weight

gain % between the health group fed on basal diet and the groups treated with 3% cherries or plumes.

The high levels of cherries, plumes and their combination from them increased the mean value of body weight gain %, as compared to the positive control group. The highest increase in the mean value of BWG% recorded for the osteoporotic group which treated with the combination of (cherries and plume) with (6%). This treatment increased the mean value of BWG% by about 77.113%, than that of the positive control group.

Table (3): Effect of cherries and plumes dried on feed intake, BWG% and femur bone/body weight% of rats suffering from osteoporosis.

Parameters Groups	Feed intake g/day	Body weight gain %	Femur bone / body weight%
Control (-ve)	19.231	$27.454^{a}$ ± 2.509	1.603 <sup>a</sup> ± 0.055
Control (+)	16.580	$10.220^{d}$ ± 3.767	$1.135^{\text{f}} \pm 0.073$
3% cherries	17.558	$10.550^{d}$ ± 2.228	$1.243^{e} \pm 0.051$
6% cherries	18.338	$14.320^{\circ}$ ± 2.110	$1.303^{de} \pm 0.068$
3% plumes	17.800	$9.131^{d} \pm 3.201$	$1.343 {}^{c  d} \pm 0.051$

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6% plumes	18.955	13.553 °	1.383 <sup>bcd</sup>
		$\pm 3.609$	$\pm 0.049$
3% (cherries and	17.330	14.498 °	1.410 <sup>bc</sup>
plumes) "1:1"		$\pm 2.659$	$\pm 0.065$
6% (cherries and	18.300	18.101 <sup>b</sup>	1.46
plumes) "1:1"		$\pm 3.551$	Т 3 <sup>в</sup>
plunes 1.1			$\pm 0.058$

Effect of dried Cherries and Plumes on rats suffering from osteoporosis

All results are expressed as mean  $\pm$  SD. BD: basal Diet

Values in each column which have different litters are significant different (p<0.05).

#### Femur bone / body weight%:

the mean value of femur bone weigh / body weiht at % of osteoporotic rats fed on basal diet showed significant decrease pI 0.05, as compared to the healthy rats fed on basal diet. All treated groups recorded significant increase in femur bone/body weight%, as compared to the positive control group. On the other hand, the highest increase in femur bone / body weight % recorded for the groups treated with high levels of the combination, followed by low levels from this combination. Li et al .,(2003) reported that , chronic use of corticosteroid is more liable to cause bone mass loss in rat cancellous bone than in the cortical bone, and mechanical properties of the cortical and cancellous bone, especially those of the latter, will also decline, to give rise to easy bone fracture at the trabecular bone in osteoporotic conditions.

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Parameters	Cholesterol	Tg	HDL-c	LDL-c	VLDL-c	
Groups	mg/dl					
Control (-ve)	91.333 <sup>f</sup>	42.667 <sup>e</sup>	54.666 <sup>a</sup>	28.133 <sup>g</sup>	8.533 <sup>e</sup>	
Control (-ve)	$\pm 6.110$	$\pm 2.516$	$\pm 2.516$	$\pm 3.354$	$\pm 0.503$	
Control (+)	136.333 <sup>a</sup>	72.666 <sup>a</sup>	37.000 <sup>e</sup>	84.800 <sup>a</sup>	14.533 <sup>a</sup>	
	$\pm 4.725$	$\pm 2.516$	$\pm 2.00$	$\pm 2.433$	$\pm 0.503$	
3% cherries	126.667 <sup>b</sup>	68.000 <sup>b</sup>	41.667 <sup>d</sup>	71.400 <sup>b</sup>	13.600 <sup>b</sup>	
570 chernes	$\pm 5.773$	$\pm 2.645$	$\pm 2.516$	$\pm 3.078$	$\pm 0.529$	
6% cherries	116.333 <sup>c d</sup>	63.333 °	45.666 <sup>bcd</sup>	58.00 <sup>d</sup>	12.667 °	
070 chernes	$\pm 4.725$	$\pm 1.527$	$\pm 2.500$	$\pm 2.749$	$\pm 0.305$	
20/ plumos	120.333 <sup>b c</sup>	62.666 °	44.666 <sup>c d</sup>	63.133 °	12.533 °	
3% plumes	$\pm 4.700$	$\pm 2.516$	$\pm 2.492$	$\pm 1.858$	$\pm 0.503$	
60/ nlumos	107.667 <sup>de</sup>	58.667 <sup>cd</sup>	47.333 <sup>bc</sup>	48.600 <sup>e</sup>	11.733 <sup>cd</sup>	
6% plumes	$\pm 4.041$	$\pm 2.309$	$\pm 2.081$	$\pm 1.637$	$\pm 0.461$	
3% (cherries and	112.667 <sup>cd</sup>	57.000 <sup>d</sup>	47.298 <sup>bc</sup>	53.933 <sup>d</sup>	11.400 <sup>d</sup>	
plumes) "1:1"	$\pm 4.932$	$\pm 2.645$	$\pm 2.051$	$\pm 2.759$	$\pm 0.529$	
6% (cherries and	100.00 <sup>e</sup>	54.333 <sup>d</sup>	49.333 <sup>ь</sup>	39.800 <sup>f</sup>	10.866 <sup>d</sup>	
plumes) "1:1"	$\pm 4.358$	$\pm 3.785$	± 1.527	$\pm 2.498$	$\pm 0.757$	

# Table (4): Effect of dried cherries and plumes on lipid profiles of rats suffering from osteoporosis.

Tg: Triglycerides HDL-c: High density lipoprotein-cholesterol

LDL-c: Low density lipoprotein-cholesterol

VLDL-c: Very Low density lipoprotein-cholesterol

All results are expressed as mean  $\pm$  SD. BD: basal Diet

Values in each column which have different litters are significant different (p<0.05). Triglycerides (mg/dl):

Treating osteoporotic groups with 3% and 6% the combination of cherries and plumes recorded more effects in reducing the mean values of serum triglyceride, as compared to the groups treated with 3% and 6% (cherries or plumes only).

The highest decrease in serum triglyceride recorded for the group which treated with the combination of cherries and plumes with high level (6%), this treatment decreased

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the mean value of serum triglyceride by about 25.229%, than that of the positive control group.

# *High density lipoprotein-cholesterol "HDL-c" (mg/dl):*

All treated groups showed significant increase pI 0.05 in serum HDL-c, as compared to the positive control group. The mean value of serum HDL-c increased gradually with increasing the levels of dried cherries, plumes and their combination.

The highest increased in serum HDL-c recorded for osteoporotic group which treated with the combination of 6% cherries or plumes, this treatment increased the mean value of serum HDL-c by about 33.332%, than that of the positive control group.

# Low density lipoprotein-cholesterol "LDL-c" (mg/dl):

Treating osteoporotic groups with the two levels of plumes (3% & 6%) recorded more effect in decreasing the level of LDL-c than that of the groups which treated with (3% & 6%) cherries, respectively. The highest decrease in serum LDL-c was found in the group treated with the high level of the combination of cherries and plumes (6%), this treatment decreased the mean value of serum LDL-c by about 53.066%, than that of the positive control group.

#### Very low density lipoprotein-cholesterol (VLDL-c):

Treating osteoporotic groups with 3% and 6% the combination of cherries and plumes recorded more effects in reducing the mean values of serum VLDL-c, as compared to the groups treated with 3% and 6% (cherries or plumes only). The highest decrease in serum VLDL-c recorded for

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the group which treated with the combination of cherries and plumes with high level (6%), this treatment decreased the mean value of serum VLDL-c by about 25.232%, than that of the positive control group.

In this respect (Filipsson *et al*, 2006) found a clear Glucocorticoid GC dose response relation with BMI, serum triglyceride, TC, and LDL-C levels.

Stacewicz et al., (2001) ;Donovan et al., (1998) ; Jennifer et al,(1998) ; tinker (1994) and Katie ,(2011) reported that, Phenolic compounds in prunes had been found to inhibit human LDL oxidation in vitro

Keith et al., (2010) reported that diets rich in fruits, e.g., cherries, and vegetables can significantly reduce CVD risk. In this randomized, placebo-controlled crossover study, we recruited 10 participants  $(38.1 \pm 12.5 \text{ y}; 8)$ females, 2 males) with BMI>25.0 ( $32.2 \pm 4.6$ ; 5 obese, 5 overweight) to consume 8 fl oz. daily of either 100% tart cherry juice or an alternate placebo beverage, for 4 weeks each with a 2 week intervening washout period. Fasting blood samples were collected at the beginning and end of each arm for measurement of biomarkers of dyslipidemia and glycemia. Total cholesterol (C) (188.1 ± 21.0 mg/dl) was not different between treatments (p>0.05) but plasma TG/HDL-C, triglycerides (TG), and VLDL were significantly reduced 10%, 17% and 15%, respectively (p<0.05) after tart cherry juice consumption. LDL-C and HDL-C were not different between treatments.

*Effect of dried cherries and plumes on kidney functions of rats suffering from osteoporosis:* 

Serum uric acid, urea nitrogen and creatinine increased significantly at p<0.05 in the positive control

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group, as compared to the negative control group. On the other hand, prednisone acetate increased the amounts of serum uric acid, urea nitrogen and creatinine by about, 58.823%, 118.320 and 121.169%, than that of the healthy rats "control negative group", respectively.

#### Serum uric acid (mg/dl):

Table (5) showed that, treating groups which were suffering from osteoporosis with 3% and 6% (cherries, plumes and their combination) led to significant decrease pI 0.05 in serum uric acid, as compared to the positive control group. The mean value of serum uric acid decreased gradually with increasing the levels of cherries, plumes and their combination. On the other hand, using dried plumes in treating the osteoporotic rats achieved more effect in reducing uric acid, as compared to cherries. The highest decrease in the mean value of serum uric acid recorded for the group treated with 6% combination of cherries & plumes, followed by the group which treated with 6% plumes. These treatments decreased the mean values of this parameter by about 33.333% & 28.518%, than that of the positive control group, respectively.

#### Serum urea nitrogen (mg/dl):

Treating groups which were suffering from osteoporosis with 3% and 6% (cherries, plumes and their combination) caused significant decrease pI 0.05 in serum urea nitrogen, as compared to the positive control group, Table (5). The mean value of serum urea nitrogen decreased gradually with increasing the levels of cherries, plumes and their combination. On the other hand, using dried plumes in treating the osteoporotic rats achieved more effect in reducing serum urea nitrogen, as compared to cherries. The

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highest decrease in the mean value of serum urea nitrogen recorded for the group treated with 6% combination of

cherries & plumes, followed by the group which treated with 6% plumes. These treatments decreased the mean values of this parameter by about 36.363% and 29.371%, than that of the positive control group, respectively.

Table (5): Effect of dried cherries and plumes on kidney functions of rats suffering from osteoporosis.

Parameters	Uric acid	Urea nitrogen	Creatinine
Groups	mg/dl		
Control ( vo)	1.700 <sup>f</sup>	21.833 <sup>f</sup>	0.633 <sup>f</sup>
Control (-ve)	$\pm 0.100$	$\pm 1.530$	$\pm 0.057$
Control(+)	2.700 <sup>a</sup>	47.666 <sup>a</sup>	1.400 <sup>a</sup>
Control (+)	$\pm 0.200$	$\pm 2.516$	$\pm 0.200$
20/ abarrias	2.433 <sup>b</sup>	43.666 <sup>a b</sup>	1.166 <sup>b</sup>
3% cherries	$\pm 0.115$	$\pm 2.309$	± 0.152
60/ abarrias	2.133 °	38.666 °	0.973 <sup>c d</sup>
6% cherries	$\pm 0.057$	± 1.527	$\pm 0.064$
3% plumes	2.266 <sup>bc</sup>	40.00 <sup>bc</sup>	1.033 <sup>bc</sup>
	$\pm 0.058$	$\pm 2.645$	$\pm 0.057$
6% plumes	1.930 <sup>de</sup>	33.666 <sup>d e</sup>	0.830 <sup>d e</sup>
	$\pm 0.060$	$\pm 2.308$	$\pm 0.051$
3% (cherries and	2.066 <sup>c d</sup>	35.667 <sup>cd</sup>	0.896 <sup>c d e</sup>
plumes) "1:1"	$\pm 0.057$	$\pm 4.509$	$\pm 0.025$
6% (cherries and	1.800 <sup>ef</sup>	30.333 °	0.753 <sup>e</sup>
plumes) "1:1"	$\pm 0.070$	$\pm 2.886$	$\pm 0.045$

All results are expressed as mean  $\pm$  SD. BD: basal Diet

Values in each column which have different litters are significant different (p<0.05). Serum creatinine (mg/dl):

Treating groups which were suffering from osteoporosis with 3% and 6% (cherries, plumes and their combination) induced significant decrease pl 0.05 in serum creatinine, as compared to the positive control group, Table (5). The mean value of serum creatinine decreased gradually

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with increasing the levels of cherries, plumes and their combination. On the other hand, using dried plumes in treating the osteoporotic rats achieved more effect in reducing serum creatinine, as compared to cherries. The highest decrease in the mean value of serum creatinine recorded for the group treated with 6% combination of cherries & plumes, followed by the group which treated with 6% plumes. These treatments decreased the mean values of this parameter by about 46.214% & 40.714%, than that of the positive control group, respectively.

Luciano, (2013) reported that Ceasing cherry concentrate consumption led to improvements in both the patient's hyperkalemia and kidney injury. Physicians should be aware of the potentially harmful side effects of cherry concentrate and approach the use of cherry extract or concentrate with caution in patients with underlying kidney disease. Robert et al, (2003) reported that, The decrease in plasma urate after cherry consumption supports the reputed anti-gout efficacy of cherries. Lachin et al., (2012) reported that cherries resulted in a significant reduction in blood glucose and urinary microalbumin and an increase in the creatinine secretion level in urea.

# *Effect of dried cherries and plumes on serum glucose and liver enzymes of rats suffering from osteoporosis:*

The mean value  $\pm$  SD of AST, ALT and ALP in rats suffering from osteoporosis which fed on basal diet increased significantly p<0.05, as compared to healthy rats fed on the same diet (115.666  $\pm$  4.041, 57.333  $\pm$  2.516 and 186.000  $\pm$  5.291 u/l) vs. (54.000  $\pm$  1.732, 57.333 $\pm$ 2.516 and 186.000 $\pm$ 5.291 u/l), respectively. Serum AST, ALT and

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ALP enzymes increased in the positive control group by about 114.196%, 243.991% and 73.292%, than that of the negative control group.

#### Serum glucose (mg/dl):

The mean value of serum glucose decreased significantly  $p \parallel 0.05$  in all tested diet, as compared to the negative control group. The data presented in Table (6) showed non-significant change in serum glucose between the osteoporotic group treated with 3% cherries and 3% plumes, the same trend was observed when compared the mean value of serum glucose between the groups treated with 6% cherries and 6% plumes.

Using the high levels and low levels from the mixtures of cherries and plumes recorded more effect in decreasing the level of serum glucose, as compared to other tested group which contains cherries or plumes only. The highest decrease in serum glucose recorded in the group treated with the high level of the mixture of cherries and plumes, followed by the groups which treated with 6% plumes and 3% combination of cherries and plumes, respectively.

#### Aspartate Amino Transferase AST (u/l):

The mean value of serum AST decreased significantly pI 0.05 in all tested diet, as compared to the negative control group. Table (6) showed gradually decrease in serum AST enzyme with increasing the levels of

cherries, plumes and their combination. The data presented in this Table revealed that, dried plumes recorded more effect in decreasing the levels of serum AST enzymes in osteoporotic groups, than that of the groups treated with cherries.The highest decrease in serum AST enzyme

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recorded in the group treated with the high level of the mixture of cherries and plumes, followed by the groups which treated with 6% plumes and 3% combination of cherries and plumes, respectively.

Table (6): Effect of dried cherries and plumes on serum glucose and liver enzymes of rats suffering from osteoporosis.

Parameters	Glucose	AST	ALT	ALP
Groups	(mg/dl)	U/L		
Control (-ve)	80.00 <sup>f</sup>	54.000 <sup>g</sup>	16.667 <sup>f</sup>	107.333 <sup>e</sup>
	± 5.00	± 1.732	± 1.527	± 6.429
Control (+)	152.667 <b>a</b>	115.666 <sup>a</sup>	57.333 ª	186.000 <sup>a</sup>
Control (+)	$\pm 3.785$	$\pm 4.041$	$\pm 2.516$	$\pm 5.291$
3% cherries	143.333 <sup>b</sup>	108.667 <sup>b</sup>	51.666 <sup>b</sup>	167.667 <sup>b</sup>
570 enerries	$\pm 3.511$	$\pm 2.309$	$\pm 2.081$	$\pm 4.932$
6% cherries	134.000 <sup>c d</sup>	98.000 <sup>d</sup>	45.000 °	158.000 °
070 enerries	$\pm 5.291$	$\pm 2.000$	$\pm 2.00$	$\pm 4.358$
3% plumes	136.333 <sup>b c</sup>	102.666 °	47.000 °	162.333 <sup>b c</sup>
570 prunies	$\pm 4.041$	$\pm 2.516$	$\pm 2.645$	$\pm 5.507$
6% plumes	126.000 <sup>d</sup> e	92.333 <sup>e</sup>	39.333 <sup>d</sup>	156.666 <sup>c d</sup>
070 prunies	$\pm 4.358$	$\pm 2.517$	± 1.154	$\pm 2.886$
3% (cherries and	128.666 <sup>c d</sup>	95.00 <sup>d e</sup>	41.666 <sup>d</sup>	154.667 <sup>cd</sup>
plumes) "1:1"	$\pm 3.511$	$\pm 1.00$	± 1.527	± 4.163
6% (cherries and	118.667 <sup>e</sup>	87.667 <sup>f</sup>	35.667 <sup>e</sup>	148.333 <sup>d</sup>
plumes) "1:1"	± 5.507	± 2.516	± 1.154	± 2.516

All results are expressed as mean  $\pm$  SD. BD: basal Diet

Values in each column which have different litters are significant different (p<0.05).

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#### Alanine Amino Transferase ALT (u/l):

Treating osteoporotic groups with two levels of cherries, plumes and their combination led to significant decrease pl 0.05 in serum ALT enzyme, as compared to the positive control group, Table (6). The mean value of serum ALT enzyme decrease gradually with increasing the levels of cherries, plumes and their combination in the diets. The highest decrease in serum ALT enzyme was found in the group treated with the high level of the mixture of cherries and plumes, followed by the groups which treated with 6% plumes and 3% combination of cherries and plumes, respectively. These treatments decreased the mean values of this parameter by about 37.789%, 31.395% and 27.326%, than that of the control positive group respectively.

#### Alkaline Phosphatase ALP (u/l):

Feeding groups of rats which suffer from osteoporosis on basal diet containing the two levels of cherries, plumes and their combination caused significant decrease p 0.05 in serum ALP, as compared to the positive control group, Table (6). The mean value of serum ALP in the group which treated with 3% cherries showed non-significant difference, as compared to the group treated with 3% plumes, the same trend was observed when compared the groups treated with 6% cherries and 6% % plumes. The highest decrease in serum ALP recorded in the group treated with the high level of the mixture of cherries and plumes, followed by the groups which treated with 6% plumes and 3% combination of cherries and plumes, respectively. The high level from the mixture decreased the mean value of serum ALP enzyme by about 20.251%, than that of the positive control group.

Lachin et al., (2012) reported that cherries resulted in a significant reduction in blood glucose and urinary microalbumin and an increase in the creatinine secretion level in urea. Extract of this plant is useful in controlling the blood glucose level. Cherries appear to aid in diabetes control and diminution of the complications of the disease. Ana et al, (2009) reported that antioxidant action of cherry juice through increased SOD (liver, blood) and Gpx (liver) activity and decreased LPO concentration. The study highlights cherry juice as a potent COX-2 inhibitor and antioxidant in the liver and blood of mice, but not in the brain. Thus, according to study, *Prunus cerasus* cv. Maraska cherry juice might potentially be used as an antioxidant and anti-inflammatory product with beneficial health-promoting properties.

Ahmed et al,(2010) reported that, reduction of serum alanine transaminase and serum alkaline phosphatase by the lower dose of prunes. There was no change in serum aspartate transaminase and bilirubin. Alteration in liver function by use of prunes may have clinical relevance in appropriate cases and prunes might prove beneficial in hepatic disease.

Effect of dried cherries and plumes on serum calcium and phosphorus of rats suffering from osteoporosis: The mean values  $\pm$  SD of serum calcium and phosphorus showed significant decrease PI 0.05 in osteoporotic rats fed on basal diet "control +", as compared to the healthy rats fed on basal diet "control -". (2.000  $\pm$  0.100 and 1.270  $\pm$  0.096mmol/l) vs. (3.266  $\pm$  0.152 and 2.733  $\pm$  0.152mmol/l), respectively. The mean values of serum calcium and phosphorus decreased in osteoporotic

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rats fed by about (38.763% and 53.530%), than that of control group fed on basal diet, respectively.

#### Serum Calcium (mmol/l):

Treating osteoporotic groups with basal diets containing (3% and 6%) dried cherries, plumes and the mixture an equal amount from them" led to significant increase p<0.05 in serum calcium, as compared to the positive control groups, Table (6). Serum calcium increased gradually with increasing the level of dried cherries, plumes and the mixture an equal amount from them". On the other hand non-significant change in serum calcium was observed between the groups treated with 3% cherries vs. 3% plumes, the same trend was found when compared the mean value of serum calcium in the groups treated with 6% cherries and plumes.

The highest decrease in serum calcium recorded for the group treated with the high level from the combination of cherries and plumes, this treatment decreased the mean value of serum calcium by about 50%, than that of the positive control group.

#### Serum phosphorus (mmol/l):

Treating osteoporotic groups with basal diets containing (3% and 6%) dried cherries, plumes and the mixture an equal amount from them" led to significant increase p<0.05 in serum phosphorus, as compared to the positive control groups, Table (7).

Serum phosphorus increased gradually with increasing the level of dried cherries, plumes and the mixture an equal amount from them". The highest decrease in serum phosphorus recorded for the group treated with the high level from the combination of cherries and plumes,

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Effect of dried Cherries and Plumes on rats suffering from osteoporosis

followed by the groups treated with 6% plumes and the 3% cherries, plumes, respectively. These treatments increased the mean value of serum phosphorus by about 61.496%, 51.181 and 43.307% than that of the positive control group.

Table (7): Effect of dried cherries and plumes on serum calcium and phosphorus of rats suffering from osteoporosis.

Parameters	Calcium	Phosphorus
Groups	mmol/L	
Control (	3.266 <sup>a</sup>	2.733 <sup>a</sup>
Control (-ve)	$\pm 0.152$	$\pm 0.152$
Control (+)	2.000 <sup>f</sup>	1.270 <sup>f</sup>
	$\pm 0.100$	$\pm 0.096$
3% cherries	2.366 <sup>e</sup>	1.586 <sup>e</sup>
	± 0.152	$\pm 0.075$
6% cherries	2.800 <sup>b</sup> c	1.820 <sup>cd</sup>
070 chernes	$\pm 0.100$	$\pm 0.020$
3% plumes	2.533 <sup>d e</sup>	1.720 <sup>d</sup>
576 pluilles	$\pm 0.115$	$\pm 0.020$
6% plumes	2.866 <sup>b</sup> c	1.920 °
070 plumes	$\pm 0.114$	$\pm 0.019$
3% (cherries and	2.667 <sup>cd</sup>	1.820 <sup>cd</sup>
plumes) "1:1"	$\pm 0.057$	$\pm 0.018$
6% (cherries and	3.000 <sup>b</sup>	2.051 <sup>b</sup>
plumes) "1:1"	$\pm 0.100$	$\pm 0.050$

All results are expressed as mean  $\pm$  SD. BD: basal Diet

Values in each column which have different litters are significant different (p<0.05).

**Pitipa, (2013)** reported that, study examined the efficacy of dietary supplementation with tart cherry on agerelated bone loss in female (5 mo) C57BL/6 mice (n=44) assigned to a baseline group or groups receiving 0, 1, 5, or 10% cherry for 90 d. Mice were weighed weekly and bone density and trabecular and cortical bone micro architecture were assessed at the end of the study. The groups receiving the 5 and 10% cherry diet had higher (P<0.05) whole body

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and tibia bone density than the control and baseline groups. The age-related decrease in trabecular bone volume of the distal femur metaphysis was prevented with cherry supplementation (5 and 10%). In these two treatment groups vertebral trabecular volume and cortical area and thickness of the femoral mid-diaphysis was increased (P<0.05) compared to the control and baseline cohorts. These findings suggest that tart cherry not only prevented age-related bone loss, but increased cortical and vertebral trabecular bone above that of baseline which is consistent with an anabolic response.

**Meredith et al ,(1980)** reported that Five month old female C57BL/6 mice (n=10/group) were either Sham operated or

ovariectomized (OVX) to induce estrogen deficiency and then randomly assign to one of three treatment groups for 28 days: Sham + Control diet (CON), OVX + CON, or OVX + 5% (w/w) cherry diet. Cherry supplementation at 5% did not prevent the OVX-induced decrease in bone mineral density (BMD) and cortical or trabecular bone in the femur, but provided some protection against trabecular bone loss in the lumbar vertebra. Finite element analyses revealed that there was no effect of cherry supplementation on biomechanical parameters within the trabecular bone of the distal femur metaphysis. These data suggest that the effects of low dose cherry supplementation are modest at best in this model and site specific.

George, (2016) reported that prunes and plums are the most effective fruit in preventing and reversing bone loss due to rich sources of phenolic and flavonoid compounds. Smith,(2007) and Bu et al.,(2008) reported that Prunes and its polyphenols decrease bone resorption by

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suppressing nuclear receptor activator for NF-kB ligand (RANKL) signaling by osteoblasts, which in-turn downregulates osteoclast differentiation and activity. The prunes polyphenols also increase osteoblast activity and function in-vitro, which was associated with up-regulation of key transcription factors and growth factors involved in osteoblast differentiation and collagen cross linking respectively and direct inhibition of osteoclastogenesis by down regulating nuclear factor for activated T cells (NFATc1) and inflammatory mediators. Arjmandi, (2001) reported that. postmenopausal women, prunes supplementation increased bone formation and decreased resorption, thus decreasing risk of osteoporotic fractures.

*Effect of dried cherries and plumes on BMD and BMC of rats suffering from osteoporosis.* The effect of two levels of cherries, plumes and their combination on bone mineral density BMD and bone mineral concentration BMC of femur bone of rats which were suffering from osteoporosis presented in Table (8). Prednisone Acetate induced significant decrease pl 0.05 in BMD and BMC, as compared to the negative control group. This treatment decreased BMD and BMC by about 35.849% and 40.068%, than that of the positive control group.

#### Bone mineral density "BMD" (g/cm2):

Treating rats which were suffering from osteoporosis with diets containing dried cherries, plumes and the mixture an equal amount from them with the levels of (3% and 6%) increased the mean values of BMD, as compared to the positive control groups. The data in this Table showed nonsignificant change between all treated groups, except the group which treated with the combination of 6% (cherries

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and plumes) "1:1". The high level from the mixture of 6% (cherries and plumes) "1:1" increased the mean value of BMD by about 47.058%, than that of the positive control group.

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Parameters	BMD	BMC
Groups	g/cm <sup>2</sup>	
Control (-ve)	0.159 <sup>a</sup>	0.342 <sup>a</sup>
Control (-ve)	$\pm 0.0055$	$\pm 0.0052$
Control (+)	0.102 °	0.205 <sup>d</sup>
Control (+)	$\pm 0.0058$	$\pm 0.0075$
3% cherries	0.125 <sup>b</sup>	0.281 °
5% chernes	$\pm 0.0101$	$\pm 0.0090$
60/ abarrias	0.131 <sup>b</sup>	0.295 °
6% cherries	$\pm 0.0076$	$\pm 0.0075$
20/	0.130 <sup>b</sup>	0.291 °
3% plumes	$\pm 0.0046$	$\pm 0.0090$

0.138<sup>b</sup>

 $\pm 0.0065$ 

0.131<sup>b</sup>

 $\pm 0.0030$ 

0.150<sup>a</sup>

 $\pm 0.0089$ 

0.313<sup>b</sup>

 $\pm 0.0152$ 

0.317<sup>b</sup>

 $\pm 0.0064$ 

0.340<sup>a</sup>

 $\pm 0.0015$ 

Table (8): Effect of dried cherries and plumes on
BMD and BMC of rats suffering from osteoporosis.

All results are expressed as mean  $\pm$  SD. BD: basal Diet

6% plumes

3% (cherries

and plumes)

and plumes)

"1:1" 6% (cherries

"1:1"

Values in each column which have different litters are significant different (p<0.05).

#### Bone mineral concentration "BMC" (g/cm2):

Treating rats which were suffering from osteoporosis with diets containing dried cherries, plumes and the mixture an equal amount from them with the levels of (3% and 6%) increased the mean values of BMC, as compared to the positive control groups. The data in this Table showed nonsignificant change between the groups which treated with 3% cherries, 6% cherries and 3% plumes. The high level of

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plumes and the low and high levels from the combinations increased the mean values of BMC significantly, as compared to the other treated groups. The high

level from the mixture of 6% (cherries and plumes) "1:1" increased the mean value of BMC by about 65.853%, than that of the positive control group. **Bahram et al,(2002)** reported that dried plums may exert positive effects on bone in postmenopausal women. Longer duration studies are needed to confirm the beneficial effects of dried plum on bone mineral density (BMD) and the skeletal health of postmenopausal women.

*Effect of dried cherries and plumes on some minerals of rats suffering from osteoporosis.* The effect of two levels of dried cherries and plumes on serum sodium, potassium (mmol/l) and zinc ( $\mu$ g/dl) of rats suffering from osteoporosis presented in Table (9).

Parameters	Sodium"Na"	Potassium"K"	Zinc "Zn"
Groups	mmol/L		µg/dl
Control (-ve)	$130.333^{a}$ $\pm 2.516$	$4.503^{a} \pm 0.105^{a}$	$89.000^{a} \pm 3.605$
Control (+)	$113.666^{\circ}$	$2.106^{f}$	$60.433^{\circ}$
	$\pm 4.041$	± 0.110	$\pm 2.676$
3% cherries	$115.666^{\circ}$ ± 3.511	$2.757^{e} \pm 0.140$	$67.333^{d}$ ± 3.511
6% cherries	119.667 <sup>b</sup>	3.123 <sup>b c</sup>	73.666 <sup>b c</sup>
	± 3.055	± 0.068	± 4.041
3% plumes	119.000 <sup>bc</sup>	2.870 <sup>d e</sup>	71.000 <sup>d</sup>
	± 3.605	± 0.147	± 2.645
6% plumes	122.666 <sup>b</sup>	$3.156^{bc}$	76.000 <sup>b c</sup>
	± 2.081	$\pm 0.051$	± 3.605

Table (9): Effect of dried cherries and plumes on some minerals of serum rats suffering from osteoporosis.

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				_
3% (cherries and	120.000 <sup>b</sup>	3.003 <sup>c d</sup>	73.666 <sup>bc</sup>	
plumes) "1:1"	$\pm 3.605$	$\pm 0.170$	$\pm 2.081$	
6% (cherries and	124.000 <sup>b</sup>	3.290 <sup>b</sup>	79.333 <sup>b</sup>	
plumes) "1:1"	$\pm 1.000$	$\pm 0.854$	$\pm 2.516$	

All results are expressed as mean  $\pm$  SD. BD: basal Diet

Values in each column which have different litters are significant different (p<0.05).

The mean values of serum sodium, potassium and zinc in healthy rats fed on basal diet were  $(130.333\pm 2.516\text{mmol/l}, 4.503\pm 0.105\text{mmol/l}$  and  $89.000 \pm 3.605 \mu\text{g/dl}$ ), while these parameters were  $(113.666 \pm 4.041 \text{ mmol/l}, 2.106\pm 0.110 \text{ mmol/l}$  and  $60.433 \pm 2.676 \mu\text{g/dl}$ ), respectively in prednisone acetate group "positive control group" fed on basal diet.

Serum sodium, potassium and zinc decreased significantly p<0.05 in the positive control group, as compared to the negative control group. *Serum sodium* "*Na*" (*mmol/l*):

The data in Table (9) showed that, all treated groups recorded significant decrease pl 0.05 in serum sodium, except the groups which were treated with 3% cherries and 3% plumes, as compared to the positive control group. Non-significant difference in serum sodium was observed between the groups which treated with the high level of cherries, the high level of plumes and the high level of the combination of cherries and plumes (6%). The most tested diet improved the mean value of serum sodium in rats suffering from osteoporosis. *Serum Potassium"K"* (*mmol/l*):

The data in Table (9) showed that, all treated groups recorded significant decrease pI 0.05 in serum potassium, as compared to the positive control group. Non-significant difference in the mean values of serum potassium was observed between the groups which treated with the high

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levels of cherries, the high level of plumes and the high level of the combination of cherries and plumes (6%). All tested diet which contains (3% & 6%) cherries, plumes and their mixture improved the mean value of serum potassium in rats suffering from osteoporosis. *Serum Zinc "Zn" \mu g/dl (mmol/l):* 

The data in Table (9) showed that, all treated groups recorded significant decrease p 0.05 in serum zinc, as compared to the positive control group. Non-significant difference in serum zinc was observed between the groups which treated with the low level of cherries and the low level of plumes (3%), on the other hand, non-significant difference in the mean values of serum zinc was observed between the groups which treated with the high levels of cherries, the high level of plumes and the high level of the combination of cherries and plumes (6%). All tested diet which contains (3% & 6%) cherries, plumes and their mixture improved the mean value of serum zinc in rats suffering from osteoporosis. Arezoo et al., )2013) reported that Adequate intake of some nutrients like zinc, vitamin A and manganese in bone formation in bone mineralization have positive effects.. Vitamin K (in form of K2) along with calcium and vitamin D induce bone fracture decrease. If intake of phosphate and sodium be more than the recommended values, they may present negative effects on bone mineralization. In conclusion, risk of

osteoporosis incidence may be diminished with an adequate and balanced diet.

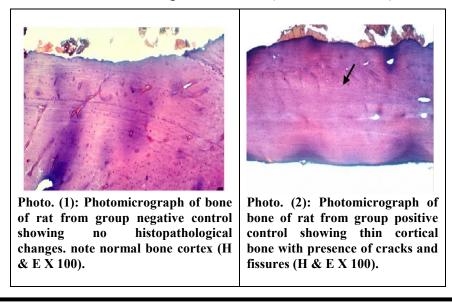
#### Histopathological examination of bones:

Microscopical examination of bone of rat from group negative control revealed no histopathological changes with

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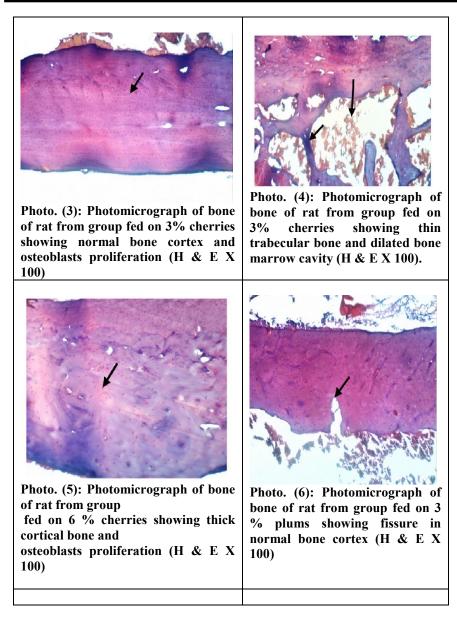
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normal bone cortex (Photo. 1). Meanwhile, bone of rat from group positive control showed thin cortical bone, thin trabecular with presence of cracks and fissures (Photo. 2). Some examined sections of bone of rat from group fed 3% cherries showed normal bone cortex and osteoblasts proliferation (Photo. 3), whereas, other sections from group fed on 3% cherries showed thin trabecular bone and dilated bone marrow cavity (Photo. 4). However, bone of rat from group fed on 6% cherries showed thick cortical bone and osteoblasts proliferation (Photo. 5). Bone of rat from group fed on 3%plums showing fissure in normal bone cortex (Photo. 6). However, bone of rat from group fed on 6% plums showed normal cortical bone, normal bone trabecular (Photo. 7) and osteoblasts proliferation (Photo. 8). Moreover, bone of rat from group fed on mix of 3% dried cherries and plums revealed no histopathological changes. note normal cortical bone (Photo. 9). Examined sections from bone of rat from group fed on mix of 6% dried cherries and plums showed osteoblasts proliferation thick bone cortex and osteoblasts proliferation (Photo. 10 & 11).



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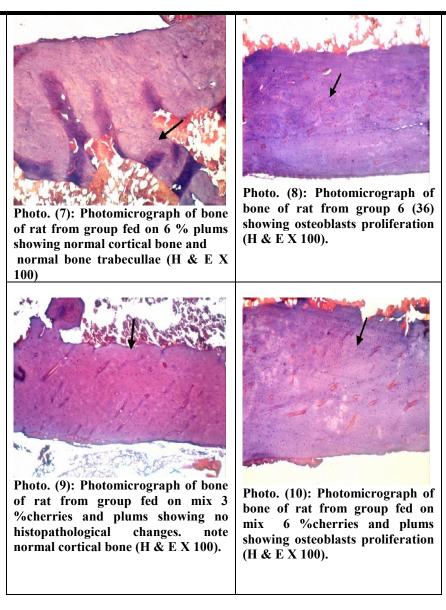
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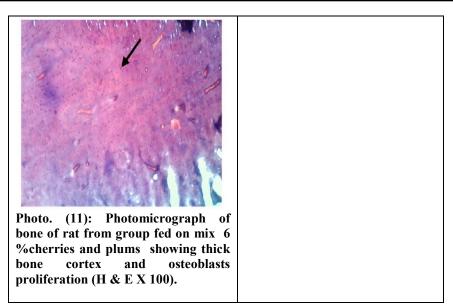
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## According to results ,we can recommend the following:

1-Nutrition education programs are needed about the important of consumption of cherries and plumes to decrease the body weight and improve glucose level ,lipid profile , kidney function and liver enzymes of people with osteoporosis.

2-Cherries and plumes should be inclouded in osteoporosis diet programs.

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