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Effect of Sumac (Rhus Coriaria L.) Herbal on Induced Osteoporosis in Female Rats

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Abstract

Sumac herbal is a nutrient-good source of mineral and phytochemical that can be used in a diet to avoid bone diseases such as rickets and osteoporosis. Thirty-six female albino rats are classified into to six groups (6 rats): first group kept as negative control group (fed on basal diet) while the other five groups injected with glucocorticoid acetate to induce osteoporosis. One group kept as positive control while the others groups treated with 10% sumac powder, 10%sumac with vitamin D, 10% sumac powder with yogurt, 10%sumac powder with vitamin D and yogurt, the study was assigned for six weeks.

The results revealed that all of the treated groups (especially sumac Vit D and yogurt) showed significant increases in bone mineral density (BMD), bone mineral concentration (BMC), calcium, osteocalcin and high density lipoprotein in comparing to positive

control group. Also the T. Cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein parameters were improved. Our results demonstrate that sumac have beneficial effects in utilizing of calcium and vitamin D in rat suffering osteoporosis.

Introduction

Osteoporosis "porous bone" is a progressive bone disease that is characterized by a decrease in bone mass and density which can lead to an increased risk of fracture often like results in broken bones, or fractures -- especially of the hip, wrist, and spine -- even from simple ***(Brian et al., 2009).*** Osteoporosis is defined by the World Health Organization ***(WHO 1994)*** as a bone mineral density of 2.5 standard deviations or more below the mean peak bone mass (average of young, healthy adults) as measured by dual-energy X-ray absorptiometry. The disease may be classified as primary type 1, primary type 2, or secondary. The form of osteoporosis most common in women after menopause is referred to as primary type 1 or postmenopausal osteoporosis, which is attributable to the decrease in estrogen production after menopause. Primary type 2 osteoporosis or senile osteoporosis occurs after age 75 and is seen in both females and males. Secondary osteoporosis results from chronic predisposing medical problems or disease, or prolonged use of medications such as glucocorticoids, when the disease is called steroid- orglucocorticoid-induced osteoporosis ***(Raisz 2005 and Armas and Recker 2012).***

Osteoporosis can also cause a vertebra to collapse. Signs of a collapsed vertebra include: Back pain, Loss of height and Kyphosis which is curvature of the spine that causes a humplike deformity ***(Armas and Recker 2012).*** There are multiple causes of bone loss and osteoporosis such as Long-term use of certain medications (e.g.: corticosteroids , Proton pump inhibitors, Anticoagulants, Barbiturates and thyroid medications) ***(Cummings and Grammack 2011 and Simonelli 2006),*** Kidney failure, not getting enough calcium, vitamin D, vitamin A, vitamin K, and magnesium, anorexia nervosa or malnutrition and alcoholism and tobacco smoking ***(Geller and Adams 2008).***

The diagnosis of osteoporosis can be made using conventional radiography and by measuring the bone mineral density (BMD).The most popular method of measuring BMD is dual-energy x-ray absorptiometry. In addition to the detection of abnormal BMD, the diagnosis of osteoporosis requires investigations into potentially modifiable underlying causes; this may be done with blood tests ***(Frost and Thomas 2012).***

The sumac (*Rhus coriaria L)* family Anacardiaceae is one of the most popular spices in Mediterranean and Arabic countries, which is obtained by crushing the dried fruits ***(Aliakbarlu et al., 2013).*** Sumac is called nutrient with all-star due to it offers nearly 20 vitamins and minerals in every serving such as vitamin K , vitamin E, calcium and folate. People tend to consume significantly more of key shortfall nutrients dietary fiber, vitamins K, and E, potassium, and magnesium in their diet through eating sumac ***(Roberts et al ., 2006 and Zainab Sajid et al.,2016).***

Sumac is rich in several vitamins B and vitamin K, with good content of vitamin C, vitamin E and potassium. Sumacs also contain phytosterols and carotenoids, such as lutein and zeaxanthin ***(Kossah et al., 2009).*** Sumac contain an oil rich in monounsaturated fatty acids (MUFA) in a water based matrix, which appears to enhance nutrient and phytochemical bioavailability and masks the taste and texture of the dietary fiber ***(Onkar et al., 2011).*** Sumac is used in traditional medicine for its antibacterial and antioxidant effect ***(Aliakbarlu et al., 2013 ; Ali-Shtayeh et al., 2013 and Kossah et al., 2013),*** antifungal and anti-inflammatory. The purpose of this study was to clarify the beneficial effect and nutritional value of sumac on induces osteoporosis rats. In addition to yoghurt and vitamins D as an enhancement for calcium absorption.

Materials and Methods

– Materials:

- Sumac *(Rhus coriaria L.)* fruits were obtained from Medical Herbs Center, Cairo, Egypt.
- Prednisone acetate as sours of glucocorticoid (GC) © was purchased from El-Gomhoria Co., Cairo, Egypt.
- Vitamin D: Vitamin D3 © was purchased from El-Gomhoria Co., Cairo, Egypt.
- Rats: Thirty six female of albino rats (Sprague Dawley) weighting 110 ±5 g, provided from of National Research Center, Cairo, Egypt, were housed in wire cages under the normal laboratory conditions.

- Methods:

-Yoghurt preparation: Lactobacillus delbrueckii subsp bulgaricus CH-2 (Chr. Hansen's Lab, (Denmark) was cultivated in 25 ml of De Man Rogosa Sharpe (MRS) broth medium at 37°C for 24 h. Streptococcus thermophilus ST-36 (Chr. Hansen's Lab) grown in 25 ml M17 broth at 40°C for 24 h. Skim milk powder was obtained from Rich Food Co. (Richmond, Virginia, USA). Human therapeutic dose of yoghurt is 2000 ml per day *(**Tamime and Robinson 1991)*** and converted to rat dose according to ***(Paget and Barnes 1964).*** The rat yoghurt dosage of 180 ml per kg body weight per day was added to the diets taking into consideration their content of protein and carbohydrate.

- Chemical analysis of raw materials:

Minerals content included (Ca, P, K, Mg, Fe & Zn) were determined according to ***Chapman & Pratt (1978).*** Total phenole and flavonoid were determined according to ***Singleton et al., (1999) and Zhishen et al., (1999).*** Antioxidant activity assayed by the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method of ***Brand-Wiliams et al., (1995)*** with some modification. The extract (0.5 and 1.0 g) in methanol (1 ml) was blended with 4 ml of 0.004% methanolic solution of DPPH. The blend was shaken strongly and left to stand for 30 min in dark and the absorbance was then measured at 517 nm.

The percent of DPPH according to the equation:

Antiradical activity =Absorbance of control-Absorbance of sample/Absorbance of control

-Biological design:

The animals were kept under observation for five days before experiment and fed on basal diet according to ***(Reeves*, *1993).*** The standard diet comprised of casein (200g/kg), cellulose (30 g/kg), corn starch (497g/kg), sucrose (100g/kg), corn oil (50g/kg), mineral mixture (100g/kg), vitamins mixture (20g/kg) and DL-methionine (3g/kg). The first group (6 rats) kept as normal control fed basal diet only. The second group (30 rats) was injected with glucocorticoid (GC) © acetate to induce osteoporosis (0.3 mg/kg, 3 times/week) ***(Schorlemmer et al., 2003)*** one group served as non treated positive control. While others groups treated with.

Group (3): Treated with sumac powder added to basal diet as 10%, sumacs

Group (4): Treated with 10% sumac powder with vitamin D© (0.1 µg/kg dissolved in ethanol daily) according to ***(Ismail et al., 1988),***

Group (5): Treated with 10% sumac powder with yogurt.

Group (6): Treated with 10% sumac powder with vitamin D with yogurt.

The study was assigned for six weeks. The food intake was calculated daily and the body weight gain was recorded weekly. Food efficiency ratio (FER) were calculated according to ***(Chapman et al., 1950).***

-Bone mineral density (BMD and bone mineral concentration (BMC):

The bone mineral content of lumbar vertebrae was measured in all groups by Energy X-ray absorptiometry (DEXA) using the Norland, small subject, resolution 0.5 × 0.5 mm, speed 60 mm/s, Host scanner 3.2, 3.2 and 1.1. The bone mineral density was expressed as gram of

mineral per unit area of bone (gr/cm2) in Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine University of Cairo.

-Biochemical analysis:

For examination of bone metabolic markers blood was taken by puncture of orbital sinus before and after performing the protocol under diethyl ether anesthesia. The blood samples immediately were centrifuged and serum samples were stored at -70 centigrade degrees until assayed. All rats were killed by overdose chloroform at the end of 7 weeks. Total calcium and alkaline phosphatase in serum were determined by spectrophotometer using commercially available test kit ***(Furuichi et al., 2000 and Fishman, 1953).*** Also, osteocalcin in serum was determined by enzyme immunoassay ***(Shoji et al., 2003).***

-**Determination of lipids**:

Total cholesterol (TC) high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG)), while (LDL-c and VLDL-c) were calculated according to the equation of ***(Richmod 1973; Lopes et al., 1977and Fossati and Prenape 1982)*** respectively.

-Statistical analysis:

One way analysis of variance (ANOVA), Duncan and Dunnett tests used to compare the means values between groups. Avalue of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Minerals composition of sumac:

From table (1) minerals content of sumac calcium, phosphor, magnesium, zinc, potassium, iron and copper were (15.45, 34.01, 32.85, 01.04, 240.13, 00.69, and 00.17 mg/100g) respectively. This result was agreed with ***Özcan and Akbulut, 2007; Kossah et al., 2009 and Kizil and Turk, 2010).***

Total phenol , Total flavonoide and scavenging effect of sumac extract on DPPH:

Data in table (2), showed that total phenol, flavonoide and scavenging effect of sumac extract on DPPH. The content of total phenol and flavonoide were (4904 and 811 mg/100g) respectively, while the DPPH radical-scavenging activity of sumac extract was (15.56 mmol/ml). The results in this study are similar to that obtained by ***Pourahmad, (2010)*** who revealed that sumac's fruit rich in flavonols, phenolic acids, hydrolysable tannins, anthocyanin and fatty acids. Sumac water extracts showed the strongest antioxidant activity ***(Bursal and Köksal, 2011 and Aliakbarlu et al.,2013).***

Nutritional effect of sumac on glucocorticoid acetate induced osteoporosis:

The results in Table (3) revealed that positive control group showed significant decrease in weight gain, food intake and FER in comparing to negative control group, result was agree with ***(Pelt, 2011)*** due to non selectivity of glucocorticoid drugs, so in the long run they may impair many healthy anabolic processes. While the other

treated groups showed significant increase in weight gain, food intake and FER in comparing to positive control group. These results were agree with ***Kossah et al., (2009)*** who mentioned that there has been the general perception that consuming foods rich in fat can lead to weight gain, and low-fat diets would more effectively promote weight control and reduce chronic disease risk.

Effect of sumac on the bone mineral density (BMD) and bone mineral concentration (BMC).

Data presented in Table (4) showed the effect of treating by sumac on BMD and BMC in glucocorticoid acetate induced osteoporosis**.** BMD and BMC are normally referring to the amount of mineral matter per square centimeter of bones. Bone density or BMD is used in clinical medicine as an indirect indicator of osteoporosis and fracture risk ***(American Academy of Family Physicians, 2012).***

The positive control group showed decrease in BMD and BMC compared to normal control group this is due to reduced bone density by Direct inhibition of osteoblast function and Direct enhancement of bone resorption ***(Richmond, 2009).*** While other treated groups showed increase in BMD and BMC compared to positive control group. The 10% sumac powder with vitamin D and yoghurt group showed the best result. These results agreed with ***(Mohammadi et al., 2010 and Shafiei, et al., 2011)*** were they reported that fruits and vegetables rich in lutein and zeaxanthin (the primary carotenoids in sumac) are associated with decreased risk of cartilage defects (early indicator of osteoporsis). There are another researches support our

results by studying the sumac or soy unsaponifiables (ASU) which is a mixture of fat soluble extracts in a ratio of about 1(sumac):2(soy). The major components of ASU are considered anti-inflammatory compounds with both antioxidant and analgesic activities ***(Dinubile, 2010).***

In vitro studies found that pretreatment of chondrocytes with ASU blocked the activation of COX-2 transcripts and secretion of prostaglandin E_2 (PGE_2) to baseline levels after activation with lipopolysaccharide (LPS). Further study revealed that ASU can also block tumor necrosis factor-α (TNF-α), IL-1β, and iNOS expression to levels similar to those in nonactivated control cultures. Additional laboratory studies suggest that ASU may facilitate repair of osteo arthritis cartilage through its effect on osteoblasts ***(Dinubile, 2010).*** Clinical support for ASU in the management of hip and knee sumac comes from four randomized controlled trials **(*Attaby et al ., 2013 and Madihi et al., 2013)*** and one meta-analysis **(*Abu-Reidah et al., 2015),*** where all studies used sumac 300 mg per day. The clinical trials were generally positive with three providing sumac support and one study showing no joint cartilage improvement compared to placebo.

Effect of sumac on calcium, alkaline phosphatase and osteocalcin in glucocorticoid acetate induced osteoporosis rats.

Osteocalcin is a non collagenous protein found in bone and dentin. As osteocalcin is produced by osteoblasts, it is often used as a marker for the bone formation process. It has been observed that lower serum-osteocalcin levels are relatively well

correlated with decrease in bone mineral density (BMD) and increase of osteoclast activity ***(Mohammad, 2012).***

The results in Table (5) revealed that positive control group showed decrease in calcium and osteocalcin while increase in ALP in comparing to negative control group this results were agree with ***(Gennari, 1993).*** The other treated groups showed that calcium and osteocalcin while decrease in ALP compared to positive control group. This was agreed with ***(Dreher and Davenport, 2013).*** As the sumac is good source of vitamin K which works in synergy with vitamin D to help regulate osteoclast production and it is also containing boron which is a trace mineral involved in bone metabolism and Vitamin D activity that reduces the amount of urinary calcium and magnesium excretion ***(Fulzele et al., 2010).***

Effect of sumac on lipids profile in glucocorticoid acetate induced osteoporosis rats.

Data presented in Table (6) showed the effect of treating by sumac on lipids profile as the positive control group showed increase in TC, TG, LDL, V-LDL and decrease in HDL compared to normal control group. while other treated groups showed decrease in TC, TG, LDL, V-LDL and increase in HDL compared to positive control group. This results was agreed with ***(Al-Jassabi & Azirun, 2010 ; Pourahmad et al., 2010 and Abu-Reidah et al., 2015)*** who found that sumac are high in fiber, and very low in carbs, two attributes that should also help promote weight loss, at least in the context of a healthy, real food based diet.

Sumacs contain monounsaturated fatty acids (MUFA)-rich fruit oil with 71% MUFA, 13% polyunsaturated fatty acids (PUFA), and 16% saturated fatty acids (SFA). As the sumac fruit ripens, the saturated fat decreases and the monounsaturated oleic acid increases ***(Marjolaine and Meyer 2009).*** The use of sumac dips and spreads as an alternative to more traditional herbs, SFA rich spreads or dips can assist in lowering dietary SFA intake**.** Additionally, several exploratory trials suggest that MUFA rich diets help protect against abdominal fat accumulation and diabetic health complications ***(Wang, et al., 2015 and Sunan and Fan 2017).***

Conclusions

The present study demonstrated the role of supplementation materials such as, sumacs, vitamin D, yoghurt and combination of them to female rats with induction of osteoporosis. Sumac with vitamin D and yoghurt are beneficial to bone health showing an in dependent association with high bone mineral density and bone mineral concentration. When evaluated together they tended to reduce bone loss and improve serum calcium, alkaline phosphates and lipid profile. It can be concluded that sumac powder has an enhancement role for calcium and vitamin D utilization in rat suffering osteoporosis. More comprehensive clinical research are needed for for expanding its utilization.

Table (1): Mineral composition of sumac (mg / 100g fresh weight basis)

Element	Composition (mg)
Calcium	15.45
Phosphor	34.01
Magnesium	32.85
Zinc	01.04
Potassium	240.13
Iron	00. 69
Copper	00.17

Table (2): Total phenol and flavonoids and scavenging effect of sumac extract on DPPH

Variables	T.Phenol mg/100g	T.Flavonoide mg/100g	DPPH mmol/ml
Sumac	4904	811	15.56

Data are means of three determinations

Table (3): Body weight gain, food intake and *FER of glucocorticoid acetate osteoporosis rats fed different sumac, vitamin D and yoghurt diets

Variables / Groups	body weight gain g/period	Food intake g/week	FER
Normal control	90.23 ± 8.34 [a]	17.19 ± 1.83 [a]	0.089± 0.001 [a]
Positive control	42.89 ± 5.46 f	10.70 ± 1.17 [c]	0.052± 0.003 [d]
10 % sumac	54.86 ± 6.72 [e]	14.89± 1.34 [b]	0.064± 0.005 [c]
10% sumac +vit. D	67.72 ± 5.60 [d]	15.83± 1.22 [b]	0.069± 0.004 [b]
10 % sumac +yoghurt	75.30 ± 8.22 [c]	16.52± 1.71 [a]	0.070± 0.002[b]
10% sumac + vit. D + yoghurt	82.42 ± 0.82 [b]	17.05± 1.81 [a]	0.071± 0.002 [b]

Mean values in each column having different superscript (a, b, c, d) are significant different at P<0.05 * FER: Food efficiency ratio

Table (4): *BMD and *BMC of glucocorticoid acetate osteoporosis rats fed different sumac, vitamin D and yoghurt diets

Variables / Groups	BMD g/cm^2	BMC g/cm^2
Normal control	0.1433± 0.0061 [a]	0.0956± 0.0031 [a]
Positive control	0.0482± 0.0041 [e]	0.057± 0.0061 [f]
10 % sumac	0.0995± 0.0039 [d]	0.0687± 0.0048 [e]
10% sumac +vit. D	0.1198± 0.0061 [c]	0.0759d± 0.0045 [d]
10 % sumac +yoghurt	0,1391± 0.0081 [b]	0.0863± 0.0041 [c]
10% sumac + vit. D + yoghurt	0.1408± 0.0091 [b]	0.0924± 0.0053 [b]

Mean values in each column having different superscript (a, b, c, d) are significant different at P<0.05

*BMD=Bone Mineral Density. * BMC= Bone Mineral Concentration.

Table (5): Calcuim, alkaline phosphatase and osteocalcin of glucocorticoid acetate osteoporosis rats fed different sumac, vitamin D and yoghurt diets

Variables / Groups	Ca (mg/dl)	Alkaline Phosphate (U/mL)	Osteocalin (mg/mL)
Normal control	11.84± 0.71 [a]	82.18± 5.31 [d]	8.52± 1.46 [a]
Positive control	7.19± 0.45 [d]	141.64± 24.49 [a]	4.98± 0.54 [e]
10 % sumac	9.75± 0.44 [c]	114.45± 12.54 [b]	5.83± 0.31 [d]
10% sumac +vit. D	10.16± 0.55 [c]	107.65± 15.07 [c]	6.09± 0.45 [c]
10 % sumac +yoghurt	10.59± 0.45 [b]	98.64± 15.13 [d]	6.52± 0.57 [b]
10% sumac + vit. D + yoghurt	11.31± 0.46 [b]	87.94± 11.65 [e]	6.82± 0.53 [b]

Mean values in each column having different superscript (a, b, c, d) are significant different at $P<0.05$

Table (6): Lipids profile of glucocorticoid acetate osteoporosis rats fed different sumac, vitamin D and yoghurt diets

Variables / Groups	TC mg/dl	TG mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
Normal control	77.30± 3.66 [e]	81.84± 3.65 [e]	41.75± 1.13 [a]	19.18± 1.23 [e]	16.37± 1.08 [e]
Positive control	119.16± 4.98 [a]	121.31± 3.69 [a]	30.48± ±1.87[d]	61.76 ±5.67 [a]	24.26 ±1.82 [a]
10 % sumac	101.29± 2.19 [b]	102.46± 4.54 [b]	35.55± 1.54 [c]	45.25± 2.44 [b]	20.49± 1.33 [b]
10% sumac +vit. D	99.28± 4.45 [c]	99.46± 2.55 [b]	37.12± 2.11 [b]	42.27± 2.01 [b]	19.89± 1.64 [c]
10 % sumac +yoghurt	79.19± 3.11 [d]	89.51± ±1.81 [c]	39.66± ±2.10 [b]	22.15± 3.11 [c]	17.90± 1.07 [d]
10% sumac + vit. D + yoghurt	75.68± 1.99 [e]	80.52± 1.96 [d]	40.61± 2.03 [a]	18.97± 3.22 [d]	16.10± 1.11 [d]

Mean values in each column having different superscript (a, b, c, d) are significant different at P<0.05

TC: total cholesterol TG: total triglyceride

HDL-C: High density lipoprotein cholesterol

LDL-C: Low density lipoprotein cholesterol

VLDLc: very low density lipoprotein cholesterol

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تأثيرعشبة السماق على اناث الفئران المصابة بهشاشة العظام

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المخلص العربى

تعتبر عشبة السماق مصدر جيد وغني بالمواد الغذائية لاحتوائه المعادن والفيتامينات والمركبات الفعالة والتي يمكن استخدامها في اتباع نظام غذائي لتجنب أمراض العظام مثل الكساح وهشاشة العظام.

وقد اجريت هذه الدراسة على ستة وثلاثين من أناث الفئران البيضاء (سبراج داولي) تم تقسيمها إلى ست مجموعات (6 فئران). المجموعة الأولى (الضابطة السالبة) والتي تغذت على الوجبة القياسية فقط، بينما المجموعات الخمس الأخرى فتم حقنها بخلات جلايكورتيكود (0.3 ملجم / كجم من وزن الفأر ، و 3 مرات / أسبوع) لاصابتها بهشاشة العظام. وتركت مجموعة واحدة كمجموعة (الضابطة موجبة) غير معالجة. بينما المجموعات الأخرى عولجت 10% بمسحوق السماق، 10% بمسحوق السماق مع فيتامين D، 10% بمسحوق السماق مع الزبادي و 10% بمسحوق السماق مع فيتامين D والزبادي، لمدة ستة أسابيع.

وأوضحت النتائج أن كل من المجموعات المعالجة أظهرت ارتفاع معنوى ملحوظ في مستوى كثافة المعادن فى العظام (BMD)، ومستوى تركيز المعادن بالعظام (BMC)، وكذلك الكالسيوم وأوستيوكالسين مقارنة مع المجموعة الضابطة الموجبة. و بينما سجلت تحسن لقيم مؤشرات مستوى الدهون في الدم تظهرنتائج الدراسة أن عشبة السماق لها آثار مفيدة للإستفادة من عنصر الكالسيوم وفيتامين د فى الفئران المصابة بهشاشة العظام .