Role of some Herbs in Improvement of Mineral Absorption, Control of Anemia and Osteopenia in Rats

Adel, A. Elmotty; Nehad R. El Tahan and Sara,S.A.

Nutrition and Food Science Dept., Faculty of Home Economics, Menoufia University, Egypt.

Abstract

The potential health benefits of Safforn **(***Crocus sativus***)**, Ginger (*Zingiber officinale*),and Cumin (*Cuminum cyminum*)powder as functional food supplement to improve Ca, P, Mg and Fe absorption as well as to prevent and treat both anemia and osteoporosis associated with deficiency in calcium and iron intake were evaluated in rats. Three experimental groups were fed diet supplemented with 10% tested herbs for 4 wk versus the control rat group fed basal diet free in iron and calcium. At the end of the experiment, rat groups fed herbs supplemented diets were characterized by significant dose-related increases in the level of serum minerals Ca (2.1 ± 0.03 to 2.58 ± 0.01 mmol/L), P (0.72 ± 0.3 to 0.93 ± 0.02 mmol/L), Mg (0.71 ± 0.01 to 0.91 ± 0.01 mmol/L) and Fe (69.11 ± 4.55 to 84.3 ± 2.12 µg/dl). In addition, there were variable increases in the measured levels of hemoglobin (11.11 ± 1.1 to 14.25 ± 1.1 g/L), hematocrit (39.15 ± 0.15 to 42.15 ± 1.27 %) and ferritin (49.55 ± 2.25 to 65.12 ± 0.15 µg/dl) in herbs fed groups in a dose-dependent fashion compared with the control group. These data suggested that Safforn **(***Crocus sativus***)** followed by Ginger (*zingiber officinale*),and Cumin (*Cuminum cyminum*)powder could provide with calcium increase, iron absorption and bioavailability of them when incorporated in daily diets and therefore, could be considered as a very effective food supplement to prevent and treat both anemia and osteoprosis, and acts as a very potent oestrogenic agent.

Key Words: Rats, Safforn, Ginger,and Cumin, Calcium and Iron Absorption, Anemia, and Osteopenia.

Introduction

Bone loss takes place throughout the aging process, but it is especially pronounced in the early-stage postmenopause women, who show a rapid decrease in bone mineral density (***Ilich and Kerstetter 2000***). Osteoporosis, which can be due to estrogen deficiency after menopause or calcium malabsorption, is a worldwide health major concern. There is a clear evidence that an adequate calcium, phosphorus and magnesium intake and bioavailability throughout lifetime is essential for the maintenance of the skeletal system and protection against bone mineral loss ***(Yang et al.; 2002 and Bronner, 2003).***

Iron-deficiency anemia is another global nutritional problem occurring as a complication of nutritional and absorption disorders and is observed frequently over ages (Makrides et al.; 2003). Shortage in dietary iron intake or absorption represents the major risk factor of the incidence of iron-deficiency anemia. Iron-deficiency has been strongly related with many human diseases including immune disorders (Kim et al.; 2002), chronic inflammation, restriction of physical performance, neurological impairment and cognitive deficits (Kriger and Schroeder 2001).

Crocus sativus Linn (family: Iridaceae) is a flowering plant in the crocus family and is commonly known as saffron. It is widely used as spice and as a coloring and flavoring agent in the preparation of various foods and cosmetics. It is native to Iran and Greece. It is now cultivated largely in Southern Europe, Tibet and other countries. In India, it is mainly cultivated in Kashmir and Uttranchal. The stigmas of the plant are mainly used for therapeutic purposes (Abdullaev, 2002). The stigmas of Crocus sativus Linn. (Saffron) are used as coloring and flavoring agents in the preparation of food in different parts of the world. Apart from its use in preparation of food, the stigmas of the plant are used for the treatment of a variety of disorders traditionally. The medicinal properties attributed to saffron are extensive. Saffron was also used as a nervine sedative, emmenogogue, in treatment of fever, melancholia and enlargement of the liver. It is also used as analgesic, diuretic, immune stimulant, interferon inducer, and for

inhibiting the thrombin formation. At low doses, it causes the stimulation of the pregnant uterus and in larger amounts it can cause constriction and spasm(Yu et al., 2007). Saffron is also a protective agent against chromosomal damage, a modulator of lipid peroxidation, and an anti seizure, for reducing blood pressure and also used in treatment of psoriasis(Kolanjiappan et al., 2002).

Ginger or ginger root is the rhizome of the plant Zingiber officinale, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family (Zingiberaceae). Other notable members of this plant family are turmeric, cardamom, and galangal. The distantly related dicots in the Asarum genus have the common name wild ginger because of their similar taste (Utpala et al., 2006)..

Ginger cultivation began in South Asia and has since spread to East Africa and the Caribbean.Ginger contains up to three percent of a fragrant essential oil whose main constituents are sesquiterpenoids, with (-)-zingiberene as the main component. Smaller amounts of other sesquiterpenoids (β-sesquiphellandrene, bisabolene and farnesene) and a small monoterpenoid fraction (β-phelladrene, cineol, and citral) have also been identified.The pungent taste of ginger is due to nonvolatile phenylpropanoid-derived compounds, particularly gingerols and shogaols, which form from gingerols when ginger is dried or cooked. Zingerone is also produced from gingerols during this process; this compound is less pungent and has a spicy-sweet aroma. Ginger is also a minor chemical irritant, and because of this was used as a horse suppository by pre-World War I mounted regiments for feaguing.Ginger has a sialagogue action, stimulating the production of saliva, which makes swallowing easier (Kumar et al., 2011).

Cumin (Cuminum cyminum), a small annual herbaceous plant that is a member of the aromatic plant family (Umbelliferae). It is a native of the Eastern Mediterranean countries and Upper Egypt, but is now cultivated in Morocco, Iran, Turkey, India, China and the Americas. The seeds of the plant are used to add flavour to spicy dishes. They are also used as an appetite stimulant and to ease severalstomach disorders.1 Its micronutrients include about 1.4 mg

iron, 38 mg potassium and 8 mg magnesium. Magnesium serves a host of functions, including promoting heart health, controlling blood pressure and aiding the absorption of calcium.2 Cumin seeds has been found to possess essential oils such as cuminaldehyde (4-isopropylbenzaldehyde), pyrazines, 2-methoxy-3-sec-butylpyrazine, 2 ethoxy-3-isopropylpyrazine, and 2-methoxy-3-methylpyrazine.3 Moreover, Cumin seeds contain possess numerous phyto-chemicals that are known to have antioxidant, carminative and anti-flatulent properties. The active principles in the cumin may increase the motility of the gastro-intestinal tract as well as increase the digestion power by increasing gastro-intestinal enzyme secretions. This spice is an excellent source of minerals like iron, copper, calcium, potassium, manganese, selenium, zinc and magnesium. It also contains very good amounts of B-complex vitamins such as thiamin, vitamin B-6, niacin, riboflavin, and other vital anti-oxidant vitamins like vitamin E, vitamin A and vitamin C.The seeds are also rich source of many flavonoid phenolic anti-oxidants such as carotenes, zeaxanthin, and lutein (Peter, 2001;Raghavan, 2007 and Sowbhagya et al., 2008).

So, this study speculated that lactoferrin might have a stimulatory effect on iron and calcium absorption. Therefore, the study examined whether tested herbs feeding of rats prevents anemia and osteoporosis throughout increase the absorption of both calcium and iron.

Material and Methods

Animals and lactoferrin-feeding protocol.

Twenty four, 6-wk-old male Sprague-Dawley rats (90 ±5 g), were housed in individual stainless-steel wire-mesh cages. Then, rats were fed diet characterized by no iron and calcium-, and magnesium-deficiency for 3 wk before the experiment as the adaptation period. The basal diet was prepared according to the formula of ***Reeves et al.; (1993)***. Vitamins mixture was prepared according to ***Campbell (1961)***. Rats were allocated randomly into four groups (n = 6). One group served as control group whereas the other three ones were the experimental groups that fed safforn, ginger,and cumin supplemented diets at the levels of 10%. Lactoferrin was supplied by DMV-International (Voegel – Holland). These doses of lactoferrin are comparable to the allowed human dietary exposure ***(Kriger and Schroeder 2001)***.Three days later; rats were fed the assigned

experimental Safforn, Ginger,and Cumin -containing diets for an additional period 4 wk. All rats received an intramuscular injection of vitamin B-12 at 0.5mg/kg body weight every week starting on the initial day of the feeding period. The rats were allowed free access to deionized water throughout the experimental period. On the final day of the experiment, all rats were anesthetized by exposure to diethyl ether then sacrificed by decapitation and whole-blood was collected by abdominal vein puncture and then femur and tibia of both sides were sampled immediately.

Measurement of biochemical parameters of anemia.

Blood was collected by tail venous puncture every week during the experimental period. Hemoglobin was determined according to ***Drabkin, (1949)***. Hematocrit was measured using a heparinized tube according to ***Mc-Inory (1954)***. Using the serum samples obtained on the final day of the experiment, serum and total iron binding capacity (TIBC) were determined by means of commercial assay kits (Sigma Diagnostic, St. Louis) according to ***Cavill's*** method ***(1986)***. Hemoglobin regeneration efficiency (HRE) were calculated according to the method and equations of ***Miller (1982)*** as follow : -

Hemoglobin Regeneration Efficiency (HRE) =

{Hb-Fe (mol)} at the end of each period – {Hb-Fe (mol)} at the beginning of each period / mol Fe consumed.

Ca, P, and Mg contents of bone and serum measurement.

Left femur from each rat was removed and then dried at 80°C for 18 h in a ceramic pot to evaluate the dry bone weight, and then ashed at 550°C for 24 h to determine the ash weight. The ashed samples were dissolved in 4 ml of 0.1 N HCl, then diluted appropriately with distilled water for atomization. After this pretreatment, the levels of Ca, P, Mg and Fe in the femur sample solution were quantified with an inductively coupled plasma emission spectrophotometer (ICPS-7000, Shimadzu, Kyoto, Japan) according to the described methods of ***Ohta et al.; (2002)***. Serum calcium and phosphorus assessed by atomic absorption spectrophotometry ***(Fraser et al.; 1986)***, whereas, serum magnesium was assessed by flame absorption spectroscopy ***(Alcock 1969)***. The diets and feces were also dried and then micropulverized. About 100 mg of diets and feces were ashed also at 550°C for 24 h. The apparent absorption of Ca, P and Mg were calculated by the following formula:

Apparent Absorption = intake – fecal excretion ***(Ohta et al.; 2002)***.

Bone mineral content and bone mineral density in femora by dual energy X-ray absorptiometry (DXA).

Bone mineral content (BMC; mg) and bone mineral density (BMD) (mg/mm^2) of the left femur of each rat were measured by dual-energy X-ray absorptiometry (DXA; model DCS-600A; Aloca, Tokyo, Japan). The BMD was calculated by BMC of the measured bone area (BA) according to ***Ohta's*** method ***(2002)***.

Statistical analysis:

Data were analyzed using one-way analysis of variance (ANOVA) followed by the student t-test for significant difference. Statistical significant difference was defined as $P < 0.05$ ***(Snedecor and Cochran 1976)***.

Results.

Body weight gain and daily total feed intake significantly (***P***<0.05) varied in the control and experimental groups (Table 1). Both body weight gain and the daily feed intake were significantly higher in the experimental groups than in the control one in a dose-dependent fashion. The increase in both weight gain and the daily-consumed food was in the range of 76.11±1.34 to 94.18± 3.1g and 15.25± 2.1 to 19.94 g/day, respectively.

Table 1 – Body weight gain and feed intake of rats fed control and herbs containing diets for 4 weeks.

Parameters	Control diet	Basal diet with 10% cumin	Basal diet with 10% ginger	Basal diet with 10% Saffron
Body Weight Gain (g)	76.11 ± 1.34	88.56 ± 0.20^a	91.35 ± 2.10^b	94.18 ± 3.10^c
Food Intake (g/day)	15.25 ± 2.1	16.88 ± 3.20^a	18.11 ± 1.10^b	19.94 ± 1.01^c

Values are expressed as the mean ± SD (n = 6)

Values with different superscript letters $^{a, b, c}$ are significantly different from the control group (***P***<0.05).

Tibia and femur BMD and minerals (Ca, P and Mg) content in the Safforn, Ginger,and Cumin fed rats were also significantly higher in a dose-dependant manner than those of control rat group that fed iron and calcium free diet (Table-2). In general, femur BMD and mineral

contents were significantly (***P***<0.05) higher than the measured values of tibia. In tibia bone, Ca, P and Mg augmented as an effect of Safforn, Ginger,and Cumin feeding in the ranges of 20.11± 0.15 to 25.85± 0.1, 10.11± 0.1 to 14.11±0.15 and 0.24 ± 0.01 to 0.55 ± 0.01 mg /100g bone, respectively. Whereas, in case of femur bone, Ca, P and Mg were increased in the range of 21.3±0.1 to 29.31±0.1, 10.88 ± 0.3 to 14.11±0.1and 0.56 ± 0.01 to 0.69± 0.01 mg /100g bone , respectively.

Table 2 – Calcium, phosphorus and magnesium concentrations and bone mineral density (BMD) in tibia and femur of rats fed control and herbs containing diets for 4 weeks.

Bone Site Profile	Control diet	Basal diet with 10% cumin	Basal diet with 10% ginger	Basal diet with 10% Saffron
Tibia	20.11 ± 0.15			
Ca (mg/100g bone)	10.11 ± 0.10	22.75± 0.20[a]	23.95 ± 0.10[b]	25.85 ± 0.10[c]
P (mg/100g bone)	0.42 ± 0.01	12.35 ± 0.25[a]	13.48 ± 0.15[a]	14.11 ± 0.15[b]
Mg (mg/100g bone)	110.30 ± 11.30	0.48± 0.01[a]	0.52 ± 0.02[a]	0.55± 0.01[b]
BMD (mg/cm^2)		121.20 ± 5.55[a]	130.50 ± 5.30[b]	139.50 ± 9.25[c]
Femu				
Ca (mg/100g bone)	21.30 ± 0.10	25.25 ± 0.20[a]	26.35 ± 0.0[b]	29.31 ± 0.10[b]
P (mg/ 100g bone)	10.88 ± 0.30	12.88 ± 0.15[a]	13.45 ± 0.30[b]	14.11 ± 0.10[b]
Mg (mg/ 100 g bone)	0.56 ± 0.01	0.59 ± 0.01[a]	0.64 ± 0.02[a]	0.69± 0.01[b]
BMD (mg/cm^2)	125.12 ± 6.77	139.25 ± 7.20[a]	148.55 ± 15.20[b]	162.10 ± 10.10[c]

Values are expressed as the mean ± SD (n = 6)
Values with different superscript letters [a, b, c] are significantly different from the control group (***P***<0.05)

The apparent and fractional absorption of Ca, P and Mg are listed in table (3). Both of the measured parameters of the three minerals (Ca, P and Mg) of the three experimental rat groups were significantly (***P***<0.05) higher than those of the control rat group in a dose-dependent manner. At the levels of 10% of Safforn, Ginger,and Cumin, the apparent calcium absorption was increased in the range of 45.25± 1.2, 49.1± 1.11 and 53.25±2.2 mg/d respectively, the apparent of phosphorus augmented in the range of 20.89±2.26, 21.75±2.35 and 23.11±1.1 mg/d, respectively and magnesium apparent absorption was

increased in the range of 4.1± 0.1, 4.25 ± 0.1 and 4.85 ± 0.3 mg/d, respectively.

Table 3 – Apparent calcium, phosphorus and magnesium absorption and their fractional absorption rates in rats fed control and herbs containing diets for 4 weeks.

Mineral Absorption	Control diet	Basal diet with 10% cumin	Basal diet with 10% ginger	Basal diet with 10% Saffron
Apparent Ca absorption, mg/d	40.33 ± 2.18	45.25± 1.20[a]	49.10± 1.11[b]	53.25 ± 2.20[c]
Fractional Ca Absorption rate, %	47.15 ± 2.10	51.35 ± 3.40[a]	60.25 ± 1.05[b]	63.5 ± 2.14[b]
Apparent P absorption, mg/d	18.25 ± 1.11	20.89 ±2.26[a]	21.75 ± 2.35[b]	23.11 ± 1.10[b]
Fractional P absorption rate, %	48.51 ± 2.25	55.25 ± 3.10[a]	60.30 ± 2.10[b]	63.15 ± 2.20[b]
Apparent Mg absorption, mg/d	3.11 ± 0.30	4.10 ± 0.10[a]	4.25 ± 0.10[b]	4.85 ± 0.30[c]
Fractional Mg absorption rate, %	50.10 ± 2.11	54.30 ± 1.05[a]	60.11 ± 1.10[b]	66.50 ± 3.35[b]

Values are expressed as the mean ± SD (n = 6)

Values with different superscript letters [a, b, c] are significantly different from the control group (***P***<0.05).

Data shown in Table (4) indicate that all of the assessed serum minerals (calcium, phosphorus, magnesium and iron) were significantly (***P***<0.05) increased as affected by Cumin, Ginger,and Safforn intake in a dose-dependent fashion whentested herbs intake level was 10%, Increased level was in the range of 2.35±0.01, 2.41±0.01 and 2.58 mmol/L respectively for calcium; 0.8± 0.02, 0.85±0.03 and 0.93±0.02mmol/L respectively for phosphorus; 6.57, 15.78 and 22.36mmol/L respectively for magnesium and 76.3±2.1,80.25±4.1 and 84.3±2.12 µg/dl respectively for iron. In addition, all serum levels of hemoglobin were also elevated in the range of 12.35±1.11, 13.15±2.36 and 14.25±1.1 g/L respectively. While, ferritin was increased in the range of 57.10±0.36, 62.3±0.1 and 65.12±0.15µg/L respectively. Hemoglobin regeneration efficiency (HRE) was increased at the range of 0.15±0.05, 0.16±0.02 and 0.18± 0.01. Hematocrit was increased at the ranges of

40.25±1.3,41.15±2.11 and 42.15±1.27% in the experimental rats fed 2.5, 5.0 and 10.0 mg/kg diet lactoferrin respectively. Hemoglobin regeneration efficiency (HRE) in the lactoferrin-fed rats was significantly higher than that of the control rat group in a dose-dependent fashion while, total iron binding capacity (TIBC) in the lactoferrin-fed rats was significantly lower than that of the control rat group in a dose- dependent fashion.

Table 4 – Serum minerals concentration and hemoglobin indices in rats fed control and herbs containing diets for 4 weeks.

Serum Profile	Control diet	Basal diet with 10% cumin	Basal diet with 10% ginger	Basal diet with 10% Saffron
Serum Ca (mmol/L)	2.10 ± 0.03	2.35 ± 0.01[a]	2.41 ± 0.01[a]	2.58 ± 0.01[b]
Serum P (mmol/L)	0.72 ± 0.03	0.80 ± 0.02[a]	0.85 ± 0.03[b]	0.93 ± 0.02[c]
Serum Mg (mmol/L)	0.71 ± 0.01	0.78 ± 0.02[a]	0.84± 0.06[b]	0.91 ± 0.01[c]
Serum Fe (µg/dl)	69.11 ± 4.55	76.30 ± 2.10[a]	80.25 ± 4.10[b]	84.30 ± 2.12[c]
HRE[1]	0.14 ± 0.01	0.15 ± 0.05[a]	0.16 ± 0.02[b]	0.18 ± 0.01[c]
Serum ferritin (µg/L)	49.55 ± 2.25	57.10 ± 0.36[a]	62.30 ± 0.10[a]	65.12 ± 0.15[b]
TIBC (µg/dl)[2]	345.10 ± 6.20	339.5± 33.50[a]	330.6 ± 25.55[a]	321.7 ± 10.50[b]
Hemoglobin (g/L)	11.11 ± 1.10	12.35 ± 1.11[a]	13.15 ± 2.36[b]	14.25 ± 1.10[c]
Hematocrite (%)	39.15 ± 0.15	41.15± 2.11[a]	41.15 ± 2.11[a]	42.15 ± 1.27

Values are expressed as the mean ± SD (n = 6)

Values with different superscript letters [a, b, c] are significantly different from the control group (***P***<0.05).

HRE[1]: Hemoglobin regeneration efficiency, TIBC[2]: total iron-binding capacity.

Discussion.

Osteoporosis is a major contributor to the high frequency of bone fracture, specially in elderly people, and therefore, many drugs have been developed to prevent osteoporosis **(Judex and Zernicke 2000 and Ohsawa *et al.*; 2002)**. In exercise therapy, the mechanical stress of exercise stimulates bone formation **(Heinonen *et al.;* 1996).** Another approach is the supplementation with nutrients such as calcium and vitamin D **(Choen and Roe 2000)**. These nutrients are required for normal bone formation, growth and development, and insufficient intake may be a cause of osteopenia. The most effective approach for preventing postmenopausal osteoporosis is hormone (estrogen) replacement therapy (HRT). However, HRT increases the risk of breast and uterine cancers. Another approach is the use of selective specific estrogen receptor modulators, which selectively act on bone and cardiovascular systems without exhibiting substantial estrogenic action in the reproductive organs(**Genant *et al.;* 1989 and Bonanni *et al.;* 2000).**

Anemia is considered as one of the most common index of malnutrition over the world and is caused by iron deficiency store (IDS) or iron-deficiency erythropoiesis (IDE) based on the screening criteria for iron-deficiency anemia **(Lin *et al.;* 2003).**

In this study, the primary cause of anemia was considered to be the feeding on iron-deficient diet (malnutrition) for a long period (3 weeks) through the adaptation feeding course before incorporation of lactoferrin together with normal load of iron and calcium into the experiment diets. The hemoglobin concentration decreased constantly during the feeding period of iron-free diets in all the rat groups. It was evident that iron deficiency contributed to this anemia, because typical signs of iron-deficiency anemia such as decreases in hemoglobin and serum iron concentrations, and increases in total iron binding capacity were observed **(Baynes and Bothwell 1990).**

Several authors have reported that iron mal-absorption is mainly caused by some of the food constituents which can be inhibitors of iron absorption and may contribute to the high prevalence of iron deficiency found. Our data indicate that milk glycoprotein “lactoferrin”-feeding prevented the development of anemia and improved hemoglobin, the hematocrit and both serum and bone iron contents in a dose-dependent manner. The final hemoglobin concentration and hematocrit in the rats fed lactoferrin were significantly higher than those in the rats fed the

control diet. Serum iron and HRE also significantly increased after lactoferrin-feeding. It has been reported that there was a high positive correlation between serum iron concentration and iron absorption **(Kim and Atallah 1993). Buchowski *et al.* (1989)** also reported a correlation between HRE and apparent absorption of iron. Feeding lactoferrin-containing diet appears to increases in total iron binding capacity as shown in our results. It seems that the effect of the ingested doses of lactoferrin were enough to stimulate iron absorption in the experimental rat groups with significant ($P<0.05$) different effect according to the ingested dose.

Observed increases in both femur and tibia bone mass as affected by lactoferrin administration suggest that lactoferrin selectively acts on bone and exhibits cooperative effect on bone mass. This finding is in accordance with that of reported by ***Choen and Roe (2000***) who stated that lactoferrin as natural glycoprotein feeding for 4 weeks plays an important role in growth and development of skeletal tissue, and prevents bone loss in male and female mice when combined with exercise.

The present data illustrate that Safforn, Ginger,and Cumin improves both bone mineral density and mineral contents of examined bones through the promotion and enhancement of Ca, P and Mg absorption and bioavailability which are mainly involved in bone tissue development (**Khare, 2004**).

In the present study, ferric pyrophosphate was used, which is a water-insoluble compound, as the iron source of the experimental diets. In this case, lactoferrin-feeding is highly suggested to decrease the pH of the cecal contents and therefore increases the iron concentration in the soluble fraction of the cecal contents. The mechanism of iron absorption via not only the small intestine, but also via the large intestine has not yet been clarified **(Ohta et al.; 1997**). However, sufficient iron is absorbed via the large intestine for recovery from iron-deficiency anemia in rats **(Ebihara et al. 1994 and Ebihara and Okano 1995).** Therefore, this study speculate that the effect of the tested herbs in increasing the absorption of iron takes place in the large intestine in rats.

There are other possible explanations for the stimulatory effect of saffron on iron absorption in the experimental rats. Thus, lactoferrin might lead to change the intestinal mucin composition and thereby increase iron absorption in the small intestine. **Joseantonio (2004)** proposed that the pathway of iron transport in the intestine consisted of several iron-binding proteins including mucin, integrin, mobilferrin and ferritin. Once the iron-mucin complex is formed, it keeps the iron in a soluble form after alkalinization in the lumen and thereby facilitates iron absorption. Second the increase in iron absorption might be due to the increase in calcium absorption that results from lactoferrin-feeding. Several authors have reported that calcium supplementation decreases iron absorption in rats and humans by an unknown mechanism.

However, **Hallberg *et al*.(1991)** speculated that calcium and iron might competitively bind to one or more substances that are important in the transcellular absorptive pathway, resulting in the inhibitory effect of calcium on iron absorption. Actually, in rats some duodenal proteins such as mobilferrin and calreticulin have affinity for both calcium and iron. Calcium binding proteins also exist in the large intestine of rats.

It could be speculated that saffron could stimulate calcium absorption via a route independent of iron absorption, such as the paracellular route, thus, saffron-feeding might reduce the inhibitory effect of calcium on iron absorption. They reported that rat fed soybean as a saffron-rich source resulted in significant increase in calcium and iron absorption. On the other hand, as regards the heme production, fermentation could stimulate it by producing the propionate salts, which promote aminolevulinate synthesis that affects heme production and synthesis **(Sheng *et al*., 2006)**.

Also, it could be observed tha higher significant correlation between BMD and apparent Ca, P and Mg absorption. Therefore, it was speculated that a long-term feeding course with saffron and ginger-containing diet would completely prevent the decrease in BMD. Moreover, it has been also shown by **Brinker (1998)** and **Chatterjee *et al*. (2005)** that saffron and ginger caused preventative effect on bone loss in OVX mice.

In this study, saffron, ginger and cumin-feeding did completely prevent the decrease in BMD of both femur and tibia seen in control rats . In addition, **Pratap *et al*., (2012)** stated that administration of ginger markedly increased serum levels of bone-specific alkaline phosphtase (B-ALK) and specific bone hormone osteocalcin "GLP" (glucagon-like peptide) resulted in significantly increase of both femur and lumbar spine BMD in postmenopausal women.

In conclusion, 10% saffron, ginger and cumin supplementation could completely prevent anemia and improves osteopenia through its potent estrogenic aand mineral absorption enhancement effects in rats.

References:

Abdullaev, F.I. (2002): Cancer Chemopreventive and Tumoricidal Properties of Saffron (Crocus sativus L.). Exp Biol Med. 227(1): 20–25.

Alcock, N. w. (1969): Development of methods for the determination of magnesium. Ann. N. y. Acad. Sci. 162, 707.

Baynes, R. D. and Bothwell, T. H. (1990): Iron deficiency. Annu. Rev. Nutr. 10:133-148.

Bonanni, B.; Guerrieri-Gonzaga, A.; Rotmensz, N.; Torrisi, R.; Pigatto, F.; Cazzaniga, M.; Mora, S.; Diani, S.; Robertson, C. and Decensi, A. (2000): Hormonal therapy and chemoprevention. Breast J. 6:317-323.

Brinker, F. (1998): Herb contraindicationsand drug interactions. Edn2. Sandy,OR: Eclectic Medical.

Bronner, F. (2003): Calcium nutrition and metabolism. Dent. Clin. North Am. 47 (2): 209-224.

Buchowski, M. S.; Mahoney, A. W. and Kalpalathika, M. P. V. (1989): Nonheme iron absorption, apparent iron absorption and hemoglobin regeneration efficiency in anemic and normal rats fed with dietary heme and nonheme iron at various levels. Nutr. Res. 9:773-783.

Cambell, J. A. (1961): Methodology of protein evaluation. Nutr. Doc. R. 10: ed. 37. WHO/FAO.,UNICEF/PAG.

Cavill, I.; Jacobs, A. and Worwood, M. (1986): Diagnostic methods for iron status. Annu. Clin. Biochem. 23, 168.

Chatterjee, S.; Datta, R.N.; Bhattacharyya, D. and Bandopadhyay, S.K. (2005): Emollient and antipruritic effect of Itch cream in dermatological disorders: A randomized controlled trial. Research Letter 37(4): 253-254.

Choen, A. J. and Roe, F. J. (2000): Review of risk factors for osteoporosis with particular reference to a possible etiological role of dietary salt. Food Chem. Toxicol. 38:237-253.

Drabkin, D. L. (1949): The standardization of hemoglobin measurement. Am. J. Med. Sci. 217-710.

Ebihara, K. and Okano, J. (1995): Comparison of bioavailability and hemoglobin repletion of ferric and ferrous iron infused into the cecum in anemic rats. Nutr. Res. 15:889-897.

Ebihara, K.; Okano, J. and Miyata, T. (1994): Comparison of ferrous and ferric iron bioavailability following rat cecal infusion. Nutr. Res. 14:221-228.

Fraser, D.; Jones, G.; Kooh, S. W. and Radde, I. C. (1986): Calcium and phosphate metabolism, in : Textbook of Clinical Chemistry, Tiez, N. W. Ed. Saunders, Philadephia. USA.

Genant, H. K.; Baylink, D. J. and Gallagher, J. C. (1989): Estrogens in the prevention of osteoporosis in postmenopausal woman. Am. J. Obest. Gynecol. 161:1842-1846.

Hallberg, L.; Brune, M.; Erlandsson, M.; Sandberg, A. S. and Hulten, L. R. (1991): Calcium: effect of different amount on nonheme- and heme-iron absorption in humans. Am. J. Clin. Nutr. 53:112-119.

Heinonen, A.; Kannus, P.; Sievanen, H.; Oja, P.; Pasanen, M. and Rinne, M. (1996): Randomised controlled trial of effect of high-impact exercise selected risk factors for osteoporotic fractures. Lancet 16:1343-1347.

Ilich, J. Z. and Kerstetter, J. E. (2000): Nutrition in bone health revisited: a story beyond calcium. J. Am. Coll. Nutr. 19(6): 715-737.

Jose-antonio. (2004): Biology, biotechnology and biomedicine of saffron.Recent Res. Devel. Plant Sci. 2127-159.

Judex, S. and Zernicke, R. F. (2000): High-impact exercise and growing bone: relation between high strain rates and enhanced bone formation. J. Appl. Physiol. 88:2183-2191.

Khare, C.P. (2004): Encyclopedia of Indian medicinal plants. Khare C.P, Germany, p 165-66.

Kim, M. and Atallah, M. T. (1993): Intestinal solubility and absorption of ferrous iron in growing rats are affected by different dietary pectins. J. Nutr. 123:117-124

Kim, S. h.; Kim, H. Y.; Kim, W. K. and Park, O. J. (2002): Nutritional status, iron-deficiency-related indices, and immunity of female atheletes. Nutrition 18(1): 86-90.

Kolanjiappan, K.; Manoharan, S. and Kayalvizhi, M. (2002): Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clinica chimica acta. 326(1-2):143-149.

Kriger, J. and Schroder, C. (2001): Iron, brain and restless leg syndrome. Sleep Med. Rev. 5 (4): 277-286.

Kumar, G.; Kathie, L. and Rao, K.V.B. (2011): Areview on pharmacological and phytochemical properties of Zingiber officinale Roscoe (Zingiberaceae), Journal of Pharmacy Research, 4(9), 2963-2966.

Lin, X.M.; Wang, Z.; Shen, X.Y.; Long, Z.; Liu, W.J.; Guo, Y.M. and Tang, Y. (2003): Iron status and effect of early iron

supplementation on sub-clinical iron deficiency in rural school-age children from mountainous areas of Beijing (China). Zhonghua Yu Fang Yi Xue Za Zhi. 37(2):115-8.

Makrides, M.; Crowther, C. A.; Gibson, R. A.; Gibson, R. S. and Skeaff, C. M. (2003): Efficacy and tolerability of low-dose iron supplements during pregnancy: a randomized controlled trial. Am. J. Clin. Nutr. 78(1):145-53.

Mc-Inory (1954): A micro hematocrit for determining the packet cell hemoglobin concentration on capillary blood. J. Clin. Path. 7:32.

Miller, J. (1982): Assessment of dietary iron availability by rat Hb repletion assay. Nutr. Rep. Int. 26:993-1005.

Ohsawa, M.; Soda, M. and Mizunma, H. (2002): Drugs in development for the treatment of osteoporosis: Nippon Rinsho 60 Suppl. 3: 538-543.

Ohta, A.; Baba, S.; Ohtsuki, M.; Takizawa, T.; Adachi, T. and Hara, H. (1997): In vivo absorption of calcium carbonate and magnesium oxide from the large intestine in rats. J. Nutr. Sci. Vitaminol. 43:35-46.

Ohta, A.; Uehara, M.; Sakai, K.; Takasaki, M.; Adlercreutz, H.; Morohashi. T. and Yoshiko Ishimi, Y. (2002): A Combination of Dietary Fructooligosaccharides and Isoflavone Conjugates Increases Femoral Bone Mineral Density and Equol Production in Ovariectomized Mice. J. Nutr. 132:2048-2054.

Peter, K. V. (2001): Handbook of herbs and spices Vol. 1.Woodhead Publishing Limited Abington Hall, Abington Cambridge, England.

Pratap, S.R.; Ritesh, J.; Rahul, M. and Prashant, T. (2012): Antidepressant activity of hydroalcoholic extract of Zingiber officinale. International Research Journal of Pharmacy. 3:149-151.

Raghavan, S. (2007): Handbook of spices, seasonings, and flavorings. 2nd Ed. CRC Press, Taylor and Francis Group, Boca Raton.

Reeves, P. G.; Nielsen, F. H. and Fahey, G. C. (1993): AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123:1939-1951.

Sheng, L.; Qian, Z.; Zheng, S. and Xi, L. (2006): Mechanism of hypolipidemic effect of crocin in rats: crocin inhibits pancreatic lipase. Eur J Pharmacol. 14; 543(1-3): 116-22.Epub 2006 Jun 2.

Snedecor, G. W. and Cochran, W. G. (1976): Statistical Methods. 6th Edn. Ames, Iowa states Univ. Press: 298.

Sowbhagya, H. B.; Rao, B. V. S. and Krishnamurthy, N. (2008): Evaluation of size reduction and expansion on yield and quality of cumin (*Cuminum cyminum*) seed oil. J. Food Engg. 84: 595-600.

Utpala, P.; Johny, A.K.; Parthasarathy, V.A.; Jayarajan, K. and Madan, M.S. (2006): Diversity of ginger cultivation in India - a GIS study, Journal of Spice and Aromatic Crop, 15 (2), 93-99.

Y u, H.S.; Lee, S.Y. and Jang, C.G. (2007): Involvement of 5-HT1A and GABAA receptors in the anxiolytic-like effects of Cinnamomum cassia in mice. Pharmacology Biochemistry and Behavior. 87(1):164-170.

Yang, C. Y.; Oh, T. W.; Nakajim, D.; Maeda, A.; Naka, T.; Ki, C. S. and Ohta, F. (2002): Effect of habitual chitosan intake on bone mass, bone-related metabolic markers and doudenum CaBP D9K mRNA in ovariectomized SHRSP rats. J. Nutr. Sci. Vitaminol. 48 (5): 371-378.

دور بعض الأعشاب فى تحسين امتصاص الأملاح المعدنية والتحكم فى أنيميا نقص الحديد فى الفئران

أ.د/ عادل عبد المعطي أحمد
أستاذ التغذية وعلوم الأطعمة
كلية الاقتصاد المنزلي – جامعة المنوفية

أ.د/ نهاد رشاد الطحان
أستاذ التغـــــذية وعلوم الأطعمـــــة
كلية الاقتصاد المنزلى - جامعة المنوفية

أ/ ساره سمير إبراهيم ابوزيد
ماجستير كلية الاقتصاد المنزلي
جامعة المنوفية

ملخص العربي

تهدف هذه الدراسة إلى تقيم العديد مـن الفوائـد الصـحية لمسـاحيق الزعفـران والزنجبيل والكمون كمكملات غذائيـه لتحسـين الكالسـيوم والفوسـفور و الماغنيسـيوم وامتصاص الحديد وكذلك لمنع وعلاج كلا من فقر الدم وهشاشة العظام المرتبطـة بـنقص الكالسيوم والحديد ، وتم تقدير ذلك على الفئران . ولتحقيق ذلك تم تغذيه ثلاث مجموعـات من الفئران على وجبات غذائيه تحتوى على ١٠ % من هذه الأعشاب لمدة أربعـة أسـابيع فى مقابل مجموعه أخرى من الفئران تتغذى على وجبات خاليه من الكالسـيوم والحديـد . وفى نهاية التجربه لوحظ أن مجموعة الفئران المغذاه على وجبـات غذائيـه غنيـه بهـذه الأعشاب قد حدث بها زياده كبيره فى نسبة المعادن فى الدم مثل الكالسـيوم (٢,١±٠,٠٣ _ ٢,٥٨±٠,٠١مليمـول/ لتـر) والفسـفور (٠,٧٢ ± ٠,٣_٠,٩٣± ٠,٠٢ مليمـول/ لتـر) والماغنسيوم (٠,٧١± ٠,٠١ _ ٠,٩١±٠,٠١ مليمول/ لتـر) والحديـد (٦٩,١١ ±٤,٥٥_ ٨٤,٣ ± ٢,١٢ مجم/ديسي) بالاضافه إلى ذلك كانت هناك زيادات متفاوته فى مسـتويات قياس الهيموجلوبين (١١,١١±١,١ _ ١٤,٢٥±١,١ جم/لتـر)، الهيماتوكريـت (٣٩,١٥ ± ٠,١٥_ ٤٢,١٥±١,٢٧ %)والفيريتين (٤٩,٥٥ ± ٢,٢٥_ ٦٥,١٢ ± ٠,١٥مجـم/ديسـي) وذلك فى مجموعات الفئران المغذاه على وجبات بها هذه الاعشاب وبالتالي فقـد أشـارت النتائج إلى إن الزعفران يليه الزنجبيل ثم الكمون يمكن ان تعمل علـى زيـاده الكالسـيوم وامتصاص الحديد وبالتالي يمكن اعتبارهم مكملات غذائية فعالـه لمنـع وعـلاج الانيميـا وهشاشه العظام وعامل استروجينى قوى جدا .