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Effect of Biscuits Supplemented with Cinnamon, Fennel, and Anise Powders on the Quality of Vital Functions in Aged Rats

Nagib, R. M.*; Sara S. Elsayed and Mona Y. A. Mostafa

Department of Home Economics, Faculty of Specifics Education, Mansoura University, Egypt

ABSTRACT

The objective of this work was to study the impact of 5% of cinnamon, fennel, and anise powder in biscuits on the quality of vital functions in 30 aged female rats, divided into 6 groups (5 rats) as following, group (1) young control group (-ve) fed on basal diet, group (2) elder control group (+ve) fed on basal diet, group (3) fed on wheat biscuit and the other groups 4, 5 and 6 were fed on cinnamon, fennel, and anise biscuits respectively during the experimental period (8 weeks). The results indicated that groups fed on biscuits supplemented with cinnamon, fennel, and anise powders showed significantly higher levels of serum HDL-c , T.Bil, SOD, CAT,GSH ,calcium and hormones progesterone, and osteocalcin, while showed significantly lower level of serum TG, TC, LDL-c , VLDL-c , AST, ALT, ALP , creatinine, urea, uric acid, MDA, hormones LH, PTH, testosterone, comparing with elder control group (+ve). Therefore, the study recommends eating products that contain cinnamon, fennel and anise powder because they have antioxidant properties and can help treat cholesterol, liver, kidney and female hormones for the elderly.

***Keywords**:* Cinnamon, Fennel, Anise, Biscuits, Lipids, Liver, Renal, Hormones, Antioxidants.

INTRODUCTION

Aging is a vital and natural phenomenon in human life, with multiple characteristics, linked to biological, social and psychological changes (Candido *et al.*, 2018). Female aging is a process involving the time of estrogen deficiency accompanied by changes in the life cycle of postmenopausal women. Changes resulting from prolonged estrogen deficiency cause disorders of the genitourinary system, changes in sexual behavior, skin turgor, bone metabolism, effects on lipid profile, and memory (Sorpreso *et al*., 2015). Aging affects arterial function, increasing the risk of cardiovascular disease (CVD) in both men and women. Women are protected from cardiovascular disease before the onset of menopause, and this is due to estrogens having beneficial effects on the arterial wall (Virani *et al*., (2021).

Medicinal plants play an essential role in medicine. They have been used in the production of medicines for thousands of years to treat health problems or prevent diseases, in addition to their use in food, whether as flavorings or in preserving food against the spread of microbes (Dar *et al*., 2017).

Cinnamon (*Cinnamomum zelanicum*) was known since long time ago, used in ancient foods and pharmaceuticals. (E)-Cinnamyl acetate is the main constituent of cinnamon, and cinnamon buds contain sesquiterpenoids (Aroraa *et al*., 2021). Cinnamon consists of components that make it work as natural antioxidants, to treat diseases associated with aging, as it mainly consists of essential oils and other compounds such as cinnamic acid, cinnamaldehyde, and cinnamates, all of which are important for treating associated diseases (Vangalapati *et al*., 2012). Cinnamon reduces oxidative stress in elderly patients, especially those with hepatic damage (Dastjerdi *et al*., 2020).

Fennel (*Foeniculum vulgare)* is widely used as a food or medicinal product. The component athenol is responsible for the aroma of fennel. It is an important spice seed crop in India with high domestic and international commercial value (Garg *et al*., 2009; Koppula and Kumar, 2013 and Solanki *et al*., 2022.). Fennel fruits contain carbohydrates, alkaloids, phytosterols, phenols, tannins and flavonoids. It is also a rich source of dietary fiber, protein, calcium, iron, magnesium and manganese. Fennel contains also a large amount of antioxidants, such as phenols and flavonoids, (Akbar, 2018). Fennel has estrogen-like activity, it's oil can reduce the symptoms of menstrual problems in women. It is also used to treat cognitive disorders, such as dementia and Alzheimer's disease (Namavar *et al*, 2003 and Nemati *et al*, 2017).

Anise (*Pimpinella anisum L.)* is an aromatic plant that has a long traditional use both in traditional medicine and in the pharmaceutical industry. Anise seeds are composed of estragole, anisaldehyde, anise alcohol, acetophenone, pinene, and limonene, but anethole is the most important volatile oil responsible for the distinctive aromatic flavor (Sun *et al*., 2019). (Rebey *et al*., 2017) revealed that anise is a good source of natural antioxidants and is also used as a food additive. The main compounds in star anise are moisture: 9-13%, protein: 18%, fatty oil: 8-23%, essential oil: 2-7%, starch: 5%, nitrogen-free extract 22-28% and crude fiber: 12-25%. The volume of the essential oil makes up about 2-3% and the anethole makes up 80-90%. The main use of anise in traditional medicine was for its carminative, antidiarrheal, and menstrual cramp effects (Anwar, 2018).

Biscuits are a favorite food item consumed by a large number of population due to its diverse tastes and low moisture content, which makes its storage last for a long

* **Corresponding author.**
E-mail address: rashamnagib@yahoo.com
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time, in addition to that it contains some vitamins and minerals, which makes it a healthy product whose manufacturing methods are being competed for (Baghel *et al*., 2020). The aim of this study was clarification the effect of biscuit fortified with cinnamon, fennel, and anise powders, on the aged female rats' health and vital functions of the body.

MATERIALS AND METHODS

Materials

Herbs: cinnamon *(cinnamomum zeilanicum*), fennel (*Foeniculum vulgare*) and anise (*Pimpinella anisum*) powders were bought from harraz market, Bab Al Khalq Square, Cairo, Egypt.

Other ingredients: such as wheat flour, eggs, corn oil, milk, baking powder, vanilla and salt bought from local market in Egypt.

Animals: 30 female Wister albino rats, 5 rats were young (6-7 month) weighing about (150±10 g) and 25 were elder (16-18 month) weighing about (290±10g), obtained from Experimental Animal House in Agricultural Research Center, Giza, Egypt**.** All the biological experimental procedures were applied in accordance with internationally guidelines for the care and use of laboratory animals. Ethical guidelines were maintained during animal handling and permission was obtained from the concerned department, Mansoura University.

Methods

Preparation of biscuits

To prepare non-herbal and herbal biscuits from (cinnamon, fennel and star anise), the form of powder was substitute with flour at a rate of 5%, 10%, and 15%, as shown in Table (A). sugar was mixed with oil until obtaining a creamy texture, and then added salt, milk, eggs and vanilla until mixed. The mixture is complete and finally, add the flour with the added baking powder with light stirring until the formation of smooth dough, and form the dough using forming molds with a thickness of 3 cm and bake the biscuits at a temperature of 200 ° C (El-Nemr and Fahmy, 1979).

Table A. Biscuits ingredients (g/100g)

Ingredients	Herbs biscuit samples								
	(control)	Cinnamon			Fennel			Anise	
		5	10	15	5	10	15	5	10	15
Wheat flour	100	95	90	85	95	90	85	95	90	85
Sugar	40	40	40	40	40	40	40	40	40	40
milk	26	26	26	26	26	26	26	26	26	26
Egg	20	20	20	20	20	20	20	20	20	20
Corn oil	16	16	16	16	16	16	16	16	16	16
Vanilla	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Backing powder	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037
Cinnamon powder	-	5	10	15	-			-		
Fennel powder	-	-			5	10	15	-		
Anise powder	-	-			-			5	10	15

Sensory evaluation of biscuits:

Sensory analysis was performed for all samples according to (Baghel *et al.,* 2020).

Biological experiment:

Animal feeding:

30 female rats were divided and grouped in well-cleaned and ventilated cages. In the first week, rats were fed basal diet (g/100g) according to NRC (1992). As shown in Table (B). Water was obtained through slotted bottles. It included in environmental conditions (temperature 24 ± 2 °C and a 12-hour light-dark cycle).

Table B. Chemical ingredients of basal diet

ingredients	g/kg Basal diet	% Basal diet
Casein	200	20
Corn starch	497	49.7
Sugar (Sucrose)	100	10
Cellulose	30	3
Corn oil	50	5
mineral admixtures	100	10
Vitamin admixtures	20	2
DL-methionine	3	0.3

Design of biological experiments:

After feeding on basal diet for seven days (adaptation period), rats were divided into 6 groups, (five in each) as follows:

- **(G1):** young female rats as a negative control group, fed a basal diet.
- **(G2):** elderly female rats as a positive control group, fed a basal diet.
- **(G3):** elderly female rats, fed the basal diet with 35% wheat biscuit.
- **(G4):** elderly female rats, fed the basal diet with 35% cinnamon biscuits.
- **(G5):** elderly female rats, fed the basal diet with 35% fennel biscuits.
- **(G6):** elderly female rats, fed the basal diet with 35% anise biscuits.

Determination of the percent of 35% of herbal biscuits in rats feeding was according to the method of (Mostafa, 2014).

Biological estimations:

Body weight gain and feed efficiency ratio (FER) were calculated according to Chapman *et al.,* (1959).

$$\textbf{Body weight gain \%} = \frac{\textbf{Final weight (g)} - \textbf{Initial weight (g)}}{\textbf{Initial weight (g)}} \times 100$$

Feed efficiency ratio (FER) = Body weight gain daily (g) / Feed intake daily (g) / 56 day

Biochemical analysis of serum:

After the end of the 56 days (experiment duration), rats were anesthetized and then slaughtered, then blood

samples were collected from the eye of the venous plexus using a capillary tube in clean, dry centrifuge tubes. Then separate the serum using a centrifuge at 4000 rpm for 10 min and kept at 18 °C until analysis El-Refai *et al., (2015).*

Lipid patterns:

- Triglycerides was determined according to Norbert, (1995).
- Total cholesterol was determined according to Jain *et al.,* (2017).
- HDL_{-C} was determined according to Lopes *et al.,* (1977).
- LDL_{-C} and $VLDL_{-C}$ were calculated by using the method of Friedewald *et al.,* (1972).

$$\textbf{LDLc = Total cholesterol - (HDL}_{\textbf{C}}\textbf{ + VLDL}_{\textbf{C}}\textbf{)}$$

$$\textbf{VLDLc = TG / 5}$$

- Athrogenic index was calculated by Cholesterol / HDL-c and LDL-c /HDL-c according to Castelli and Levitar, (1977).

Liver function:

- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to the method described by Reitman and Frankel, (1957).
- The liver function ability of serum bilirubin (TB) was tested according to the method of Jaeschke *et al.,* (2003).
- The activities of serum alkaline phosphatase (ALP) enzyme was colorimetrically estimated according to the method of DGKC, (1972).
- Serum albumin (Alb) was determined according to the method of Al-Amoudi, (2017).

Kidney function:

- Serum urea was determined according to Malhotra, (2003).
- Uric acid and creatinine were determined according to the methods described by Tietz, (1995), and Barthes, *et al.,* (1972).

Serum Ca and P: Calcium and phosphorous were assessed using a colorimetric method in serum according to Tietz, (1970).

Antioxidant activity:

- The activity of tissue antioxidant enzyme superoxide dismutase (SOD) was estimated according to the method described by Burda, and Oleszek (2001).
- The activity of antioxidant enzyme catalase (CAT) was determined according to the method of Beutler, (1963).
- Lipid peroxide was estimated by spectrophotometry of lipid peroxide, malondialdehyde (MDA) by Ohkawa *et al.* (1979)

The non-enzymatic antioxidant, glutathione (GSH) was determined by the method Jollow *et al.,* (1974).

Hormones*:*

- Progesterone was estimated by the method of Wajchenberg *et al*., (1979), and testosterone estimated by method of Swerdloff, (2008).
- LH was determined using the method of Maruyama *et al.,* (1987).
- Serum PTH was estimated through radioimmunoassay (RIA) according to Deftos *et al*., (1989).
- Serum osteocalcin was determined using ELISA kit by the method of Rosenquist *et al*., (1995).

Statistical analysis:

The calculated data occurred by analysis of variance ANOVA and follow up test LSD the expressed as mean ± standard deviation (mean ± SD) by CoSTATE (2004) computer software according to Gomez and Gomez, (1984).

RESULTS AND DISCUSION

Biscuits flavored with different levels of cinnamon, fennel, and anise powders:

Table (1), showed the values of taste, odor, texture, color, crust appearance to develop acceptable sensory properties of biscuit product. The herb proportions in biscuits was 5, 10 and 15 of cinnamon, fennel and anise powder.

Table 1. Sensory evaluation scores of wheat biscuits flavored with different levels of cinnamon, fennel, and anise powders.

Variable / Biscuits	Taste	Odor	Texture	Color	Crust appearance	Mean scores
Control	ab 7.6±0.11	ab 7.9±0.22	a 7.5±0.14	b 7.6±0.12	bc 7.5±0.34	7.6
Cinnamon 5%	a 8.1±0.54	a 8.0±0.49	a 7.4±0.28	a 8.8±0.35	a 8.0±0.32	7.9
Cinnamon 10%	bc 7.4±0.24	ab 7.7±0.25	a 7.4±0.16	a 8.3±0.19	bcd 7.4±0.22	7.8
Cinnamon 15%	bc 7.5±0.25	b 7.5±0.26	a 7.4±0.24	bc 7.2±0.31	bc 7.7±0.25	7.5
Fennel 5%	bc 7.5±0.26	c 6.7±0.11	abc 7.0±0.43	cd 6.8±0.38	bcd 7.4±0.25	6.9
Fennel 10%	de 6.6±0.15	ef 5.7±0.21	de 6.4±0.28	ef 6.2±0.45	ef 6.9±0.24	6.7
Fennel 15%	f 5.6±0.27	f 5.4±0.24	e 6.1±0.44	g 5.6±0.14	f 6.7±0.47	6.6
Anise 5%	cd 7.0±0.34	cd 6.8±0.25	bcd 6.7±0.32	bc 7.2±0.34	f 6.7±0.18	7.1
Anise 10%	cd 7.0±0.45	cd 6.4±024	b-e 6.6±0.36	cde 6.7±0.19	ef 6.9±0.24	6.4
Anise 15%	e 6.5±0.19	de 6.0±0.23	a 7.4±0.17	fg 5.7±0.43	cd 7.3±0.15	5.9

Numbers are mean ± standard deviation. Different superscript letters in the same column indicate mean values differ significantly ($p \leq 0.05$).

Data cleared that adding 5% powder of cinnamon, fennel, and anise showed significant increase ($P< 0.05$) for all sensory acceptability, comparing with 10, and 15% of powder. So the percent of 5% was chosen to be the best one according to the sensory evaluation. At the link with our results, (Ng and wan Rosli, 2014) cleared that, addition of

small amounts of cinnamon powder can developed flavor and taste, However the addition of higher amounts of cinnamon powder led to lower scores for sensory acceptable due to its brownish color and strong flavor. Another study by (Djidjor *et al.*, 2020) confirmed that the biscuit comprising 80% wheat flour, 5% cinnamon powder and 15% coconut flour was highly accepted by consumers compared with the higher levels. Also On contrast, (Saber and Eshra, 2019) study didn't show any significant differences in the organoleptic contributes of biscuit fortified with different ratios of fennel seeds and its oil comparing with the control sample. Regards anise, (Lotfy *et al.*, 2018) found that adding 3% of anise have the ability to increase the overall acceptability in baking products compared to other concentrations.

Effect of feeding on biscuits supplemented with cinnamon, fennel, and anise powders on final weight, body weight gain, feed intake and feed efficiency ratio of aged rats:

Data represented in Table (2), showed final weight ,weight gain (g), weight gain (%), feed intake and feed efficiency ratio, in aged female rats fed on basal diet and aged female rats fed on biscuit supplemented with 5% powder of cinnamon, fennel, and anise

+ve elder control group (G2) showed a significant increase at P< 0.05, in the final weight and feed intake, while showed a significant decrease at P< 0.05, in each of body weight gain (g), body weight gain (%), and feed efficiency ratio, compared with the -ve young control group (G1).

By reviewing all biscuits groups, results cleared that, feeding on wheat biscuit (G3), showed non-significant differences at P< 0.05, in each of final weight, body weight gain (g), body weight(%), feed intake, and feed efficiency ratio, compared with +ve elder control group.

On the other side, feeding on cinnamon powder biscuit (G4), fennel powder biscuit (G5), and anise powder biscuit (G6), showed a significant increase at P< 0.05, in each of, body weight gain (g), body weight(%), feed intake, and feed efficiency ratio, compared to +ve elder control group.

It can be concluded that adding cinnamon, fennel, and anise as a powder to the product, could improve feed intake, and feed efficiency ratio, of aged female rats, in this respect, (El-Kherbawy *et al*., 2009), cleared that cinnamon have the ability to improve final weight, weight gain%, and feed efficiency ratio in rats. In contrast to our results, (Aboelnaga, 2015) showed that the increase of anise gradually by (2, 4 and 6%), caused significant decrease in body weight gain%, compared to negative and positive control groups.

Table 2. Effect of biscuits supplemented with cinnamon, anise and fennel powders on final weight , body weight gain, feed intake and feed efficiency ratio :

Variable Groups	Initial weight (g)	Final weight (g)	body weight gain (g)(gm)	Body weight gain(%)	Feed intake	Feed efficiency ratio
G1: (-ve) young control	b 155.00±6.56	d 214.00±7.81	a 59.00±3.46	a 38.07±2.48	f 20.74±0.75	a 0.047±0.003
G2:(+ve) elder control	a 250.33±3.51	c 279.00±6.56	cd 28.67±5.51	d 11.44±2.22	e 29.96±0.51	ef 0.016±0.003
G3: Wheat Biscuits	a 257.33±4.16	c 283.00±5.57	d 25.67±1.53	d 9.98±0.43	b-e 30.52 ±0.53	f 0.014±0.001
G4: Cinnamon biscuit	a 256.00±4.58	a 309.67±4.16	a 53.67±.2.52	b 20.00±1.54	a 31.74±0.55	bc 0.028±0.002
G5: Fennel biscuit	a 256.67±2.52	ab 303.00±4.36	b 46.33±2.52	bc 18.02±0.92	abc 31.45±0.35	cd 0.024±0.001
G6: Anise biscuit	a 254.67±3.21	a 308.33±0.58	a 53.67±2.89	b 21.07±1.42	ab 31.50±0.20	b 0.028±0.002

Numbers are mean ± standard deviation of 5 rats in each group. Different superscript letters in the same column indicate mean values differ significantly ($p \leq 0.05$).

Effect of feeding on biscuits supplemented with cinnamon, anise and fennel powders on serum lipid profile levels of aged rats:

The obtained data in Table (3) revealed that, there was a significant increase (P< 0.05) in (+ve) elder control in CH, TG, LDL, VLDL, HDLc, and LDLc/HDLc, with values (85.00, 115.33, 34.27, 23.07, 3.08, and 1.24) mg/dl, respectively, compared with (-ve) young control, except (HDL) level, showed non-significant difference (P< 0.05), compared with (-ve) young control (G1).

Regards all biscuits group, wheat biscuit (G3) showed non-significant differences (P< 0.05), in CH, TG, HDL, LDL, but showed an increase at P< 0.05 in both LDL, HDLc, and LDLc/HDL, compared to +ve elder control group.

On the other hand, Data also showed that each of cinnamon powder biscuit (G4), and anise powder biscuit (G6), showed significant decrease (P< 0.05) in levels of CH, TG, LDL, VLDL, HDLc, and LDLc/HDLc, while showed a significant increase in HDL serum level, compared to +ve elder control group, on the other hand fennel powder biscuit (G5), showed a significant decrease (P< 0.05) in levels of CH, TG, LDL, VLDL, and LDLc/HDL, while showed non-significant differences (P< 0.05) in HDL serum level, compared to +ve elder control group.

It could be observed that the addition of cinnamon, fennel, and anise powders were better, in improving all aged female rats' serum lipid levels, compared to the wheat biscuit, and basal diet in +ve elder control group.

(Koofee *et al*., 2019) at the link with our results, that there is a significant decrease in in HDL-C level in aged women when compared with young women, and the significant changes in the proportions of fat considered a risk factor for blood vessel and heart disease.

In agreement with our findings, (Abd Elwahab *et al.*, 2021), showed that the supplementation with fennel seeds increased HDL level. Another study by (Naderi *et al.*, 2019)

cleared that treatment with fennel seeds reduced significantly the levels of TG, total cholestserol, and LDL, but increased the level of HDL, this in agreement with our study results. Also (Al Jamal, 2009) reported that 4 weeks of cinnamon improved the mean of high density lipoprotein (HDL-C), in hyperglycemic rats.

Table 3. Serum lipid profile of experimental rats fed on biscuits supplemented with cinnamon, fennel, and anise powders.

Variable Groups	CH (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic indices (Cholesterol/HDLc) (mg/dl)	Atherogenic indices (LDLc/HDLc) (mg/dl)
G1: (-ve) young control	d 44.33 ±4.16	e 66.33 ±4.04	abc 30.67 ±2.52	f 2.00 ±0.40	e 13.27 ±0.81	f 1.44 ±0.11	f 0.06 ±0.01
G2: (+ve) elder control	a 85.00 ±4.58	ab 115.33 ±5.51	c 27.67 ±2.08	b 34.27 ±2.00	ab 23.07± 1.10	b 3.08 ±0.13	b 1.24 ±0.09
G3: Wheat Biscuits	a 93.00 ±4.58	a 123.33 ±7.57	c 27.00 ±2.00	a 41.20 ±5.41	a 24.67 ±1.51	a 3.46 ±0.38	a 1.54 ±0.30
G4: Cinnamon biscuit	cd 51.00 ±5.57	de 75.00 ±6.00	ab 33.33 ±3.79	f 2.73 ±2.05	de 14.93 ±1.30	ef 1.53 ±0.08	ef 0.07 ±0.06
G5: Fennel biscuit	c 58.67 ±5.51	d 82.00 ±5.00	abc 32.33 ±1.53	e 9.93 ±5.13	d 16.40 ±1.00	e 1.80 ±0.24	e 0.31 ±0.17
G6: Anise biscuit	c 55.67 ±5.69	d 78.67 ±5.51	ab 33.33 ±2.52	ef 6.60 ±2.95	d 15.73 ±1.10	ef 1.67 ±0.11	ef 0.19 ±0.08

Numbers are mean ± standard deviation of 5 rats in each group. Different superscript letters in the same column indicate mean values differ significantly ($p \leq 0.05$).
biscuit; CH = cholesterol; TG = Triglyceride; HDL-C = High-density lipoprotein cholesterol LDL-C = Low-density lipoprotein cholesterol.

Effect of feeding on biscuits supplemented with cinnamon, anise and fennel powders on serum liver enzymes of aged rats:

Our results in Table (4), indicated that +ve elder control group, fed on the basal diet showed a significant increase (P< 0.05) in each of rats' ALT, AST, ALP, T.Bil serum levels, compared to –ve young control group (G1), while showed a significant decrease (P< 0.05) in ALb serum level (3.03) g/dl.

Concerning biscuits groups, data obtained cleared that groups fed on wheat biscuit (G3), showed non-significant differences (P< 0.05), in each of ALT, AST, ALP, T.Bil, and ALb level, comparing to the +ve elder control group .

On the other hand, groups fed on cinnamon powder biscuit (G4), fennel powder biscuit (G5), and anise powder biscuit (G6), showed a significant decrease at P< 0.05, in ALT, AST, ALP, T.Bil serum levels, compared to +ve elder control group, while showed a significant increase at P< 0.05, in ALb serum level.

Our findings indicated that feeding on biscuit supplemented with cinnamon, fennel and anise, enhanced liver blood levels, unlike the wheat biscuit and the basal diet in +ve elder control group.

(Shekarchizadeh-Esfahani *et al.*, 2021) noticed that cinnamon supplementation had no significant effect on liver enzymes in adults of both sexes, except ALT levels, which was significant at doses of <1500 mg / day for 12 weeks. (Adeoye *et al.*, 2019) confirmed that cinnamon powder at a concentration of 5% had a detrimental effect on the liver enzymes AST and ALT. (Ezz-Eldin *et al*., 2011) concluded that Medicinal plants such as fennel play a good role in reducing the risk of fatty liver, atherosclerosis and regulating diet quality in adult female rats. On contrast, A National Toxicology Program study (Bristol, 2011) report showed that prolonged use of fennel seeds caused liver injury by promoting programmed cell death, alteration of cell membranes, and marked hepatocyte necrosis and apoptosis in a study of a number of women, in another study by (Mannaa *et al*, 2015) indicated that fennel seed extract could significantly decrease serum AST and ALT due to hepato protective activity. As for anise, (Ibrahim's 2017) study showed that though anise benefits such as improving liver function tests by reducing important factors, including AST, total and direct bilirubin However the study concluded after examining its effect on liver .

Table 4. Serum liver enzymes of experimental rats fed on biscuits supplemented with cinnamon, fennel, and anise powders.

Variable Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	T.Bil (mg/dl)	ALb (g/dl)
G1 (-ve) young control	e 36.00 ±7.00	e 90.33 ±15.70	e 307.67 ±27.01	e 0.34 ±0.06	a 4.02 ±0.11
G2 (+ve) elder control	ab 71.33 ±3.06	ab 188.67 ±5.51	ab 572.33 ±23.54	ab 0.83 ±0.04	d 3.03 ±0.11
G3: Wheat Biscuits	a 77.67 ±4.04	a 200.67 ±10.26	a 620.33 ±38.07	a 0.89 ±0.04	d 2.86 ±0.12
G4: Cinnamon biscuit	de 44.00 ±4.58	d 119.00 ±21.28	d 366.00 ±39.51	de 0.42 ±0.07	ab 3.82 ±0.13
G5: Fennel biscuit	d 50.00 ±4.00	c 145.33 ±15.57	d 411.67 ±29.02	d 0.52 ±0.08	b 3.69 ±0.10
G6: Anise biscuit	d 47.00 ±6.00	cd 136.33 ±21.59	d 392.00 ±40.51	d 0.49 ±0.08	b 3.75 ±0.13

Numbers are mean ± standard deviation of 5 rats in each group. Different superscript letters in the same column indicate mean values differ significantly ($p \leq 0.05$).
ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; T.Bil: Total bilirubin; Alb: Albumin.

Effect of feeding on biscuits supplemented with cinnamon, fennel, and anise powders on renal function of aged rats:

Data of renal function (creatinine, urea and uric acid) of experimental rats fed on cinnamon, fennel, and anise biscuits, are tabulated in Table (5).

Our results cleared that both of +v elder control group (G2), and wheat biscuit group (G3), showed a significant increase (P< 0.05) in serum creatinine, urea, and uric acid, compared with –ve young control group (G1). In this regard, (Zhang *et al*., 2019) cleared that creatinine is an indicator of the body's filtration rate. an increased urea level is also a risk factor for high blood pressure. (Olukiran *et al*., 2018), found that aged female rats recorded the highest level of creatinine in plasma compared to young rats, and this may be due to aging factors associated with a decrease in glomerular filtration rate, on the contrary, its concentration was highest in young rats compared to the elderly.

(Karakaya and Esrefoglu, 2022) discussed in a comparison of kidney functions between young and old rats, where the number of sclerotic glomeruli in old kidneys was higher than that in young ones. While the average number of healthy glomeruli in the aged kidney was lower than that of the young kidney. This supports the idea that the filtering capacity of the kidneys decreases with age.

Groups fed on cinnamon powder biscuit (G4), fennel powder biscuit (G5), and anise powder biscuit (G6), showed a significant decrease (P< 0.05) in creatinine, urea, and uric acid, compared to +v elder control group (G2).

We observed that, cinnamon, fennel, and anise biscuits were the best, in improving all renal function efficiency, compared with the wheat biscuit.

(Cicero *et al.*, 2015) demonstrated that serum uric acid (SUA) has deleterious effects on brain structure and function by affecting the viability of neurons and their ability to form synaptic connections in an in vitro model of Alzheimer's dementia (AD). On the contrary, (Bowman *et al*., 2010), (McFarland *et al*., 2013) , and (Liu *et al*., 2017) revealed the antioxidant effect of serum uric acid (SUA) similar to that of ascorbate, an important antioxidant so low levels can reduce protection against oxidative stress. If the level of uric acid in the blood (SUA) is more than 60 micrograms / ml for women, it is considered a risk factor, and vice versa if it is lower than this level Lijun *et al*, (2020). In disagreement with our results, (El-yamani 2011) and (Safdar *et al*., 2016) reported that Consuming cinnamon did not cause any significant changes in urea, creatinine and electrolytes in the blood of adult female mice, which does not harm the formation of healthy kidneys and makes them safe.

On the other hand, (Arzoo and Parle2017) confirmed the role of athenol as a major component in fennel seeds has been shown to have a protective effect on the kidneys by reducing inflammation. According to (Bekara *et al*., 2016), anise (*Pimpinella anisum L*.) has a good effect on kidney function due to its antioxidant properties. (Nair *et al*, 2018) cleared that Pimpinella anisum L. have the ability to contribute to protection against renal failure by improving the structure of glomeruli and kidney tubules in the anise-treated group. (Ibrahim, 2017) found that anise seeds have an improving effect on the functions of kidney enzymes, especially urea. This in agreement with our current study, the group treated with anise powder biscuits had a significant reduction in the level of urea compared to the control group at P< 0.05.

Table 5. Serum renal function of experimental rats fed on biscuits supplemented with cinnamon, anise and fennel powders.

Variable Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
G1 (-ve) young control)	0.52^{e}±0.06	22.00^{f}±4.00	0.55^{f}±0.09
G2 (+ve) elder control)	0.96^{ab}±0.03	54.00^{ab}±6.56	1.54^{ab}±0.06
G3: Wheat Biscuits	1.06^{a}±0.08	58.33^{a}±9.45	1.64^{a}±0.07
G4: Cinnamon biscuit	0.63^{de}±0.07	31.67^{de}±3.79	0.74^{e}±0.08
G5: Fennel biscuit	0.69^{d}±0.05	37.33^{de}±2.52	0.87^{e}±0.13
G6: Anise biscuit	0.67^{d}±0.06	31.33^{e}±3.51	0.81^{e}±0.13

Numbers are mean ± standard deviation of 5 rats in each group. Different superscript letters in the same column indicate mean values differ significantly (p ≤ 0.05).

Serum calcium and phosphorus of aged rats fed on biscuits supplemented with cinnamon, fennel, and anise powders.

Indicators of calcium and phosphorus in Table (6) cleared that feeding on basal diet in +ve control group (G2), and wheat biscuit (G3), showed a significant decrease at P< 0.05 in serum calcium level, compared to –ve young control group (G1), but they showed a significant increase at P< 0.05 in phosphorus serum level.

Groups fed on cinnamon powder biscuit (G4), fennel powder biscuit (G5), and anise powder biscuit (G6), showed a significant increase (P< 0.05), in serum calcium level, but showed a decrease in serum phosphorus compared to +ve control group.

Table 6. Serum calcium and phosphorus of experimental rats fed on biscuits supplemented with cinnamon, anise and fennel powders.

Variable / Groups	Calcium(mg/dl)	Phosphorous (mg/dl)
G1 (-ve) young control	10.70^{a}±0.19	5.11^{e}±0.16
G2 (+ve) elder control	8.36^{de}±0.07	6.56^{ab}±0.16
G3: Wheat Biscuits	8.21^{e}±0.11	6.67^{a}±0.12
G4: Cinnamon biscuit	9.83^{b}±0.35	5.43^{d}±0.16
G5: Fennel biscuit	9.45^{b}±0.39	5.65^{d}±0.16
G6: Anise biscuit	9.65^{b}±0.34	5.54^{d}±0.20

Numbers are mean ± standard deviation of 5 rats in each group. Different superscript letters in the same column indicate mean values differ significantly (p ≤ 0.05)

Our findings led to that supplementing biscuits with cinnamon, fennel, and anise were the best in improving calcium, and phosphorus serum, compared with +ve elder control group.

Based on results, (El-kutry *et al*., 2009) found that addition of fennel seeds in the diet leads to a significant increase in the concentration of calcium in the blood, heart and leg serum, with an agreement with (Bhavsar *et al*., 2022) who reported that Fennel contains a good percentage of phosphate and calcium, in addition to other vitamins and mineral elements that build and maintain bone structure and strength. At the same side, (Mahmoudi *et al*., 2013) showed that fennel extract has a protective effect against osteoporosis.

Numerous studies have confirmed the effective effect of anise (*Pimpinella Anisum L.)* in treating age-related health problems in women, and with regard to bone problems, especially after menopause. Hassan and Saed (2011) stated that dietary calcium moderately reduces the rate of cortical

bone loss in old age, and suggested that an oral dose of aniseed over a 20-week period could prevent estrogen-deficient bone loss and improve bone efficiency in ovariectomized rats.

Serum antioxidant enzymes activity (SOD, CAT, GSH) and free radical (MDA) of aged rats fed on biscuits supplemented with cinnamon, anise and fennel powders.

Table (7), showed levels of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH), and free radical, Malondialdehyde (MDA) as antioxidant indicators. Data illustrated that feeding on wheat biscuit in elder rats (G3), and basal diet in +ve elder control rats (G2) showed a significant decreases (P< 0.05), in each of, SOD, CAT, and GSH levels, while they showed an increase in MDA level compared to –ve young control rats (G1). This is in agreement with (Bilgin *et al*., 2020), which proved that, CAT, SOD, and GSH levels are affected with aging, also (Kozakiewicz *et al*., 2019), cleared that the activity of Zn,Cu-superoxide dismutase (SOD-1),catalase (CAT), and glutathione peroxidase (GSH-Px) decreases significantly in elderly people compared to the younger.

results also cleared that, feeding on cinnamon powder biscuit (G4), fennel powder biscuit (G5), and anise powder biscuit (G6), showed a significant increase at P< 0.05 in SOD, CAT, and GSH levels, but decreased in MDA level compared to +ve elder control group .

It could be concluded from the previous results that the addition of cinnamon, fennel, and anise to biscuits improved its antioxidant activity, because they were more able to raise positively the levels of SOD, CAT and GSH in the blood, they could also decreased positively the level of the free radical MDA.

Results of our experiment confirmed clearly that cinnamon had important role as antioxidant agent, it improved the level of SOD, CAT, GSH and MDA. Compounds isolated from cinnamon such as flavonoids and polyphenols have free radical–scavenging activities and antioxidant properties and this at the link with (Ranasinghe *et al*., 2013), also (Qin *et al*., 2010), reported that constitutes of cinnamon like cinnamaldehyde, phenols, terpenes are responsible for antioxidant activity. Study of (Noori *et al*., 2012) reported that Cinnamon extract showed antioxidant activity in liver tissue. In (Borzoei *et al*., 2018) study about the comparison between cinnamon supplementation with placebo in women With PCOS, their research extracted an effective effect of cinnamon on antioxidants and blood lipids during 8-week intake compared to baseline values. In harmony with our results, (Shanmugam and Naidu, 2000), (Eidi *et al*., 2012) and (Morgan *et al*., 2014) confirmed the antioxidant effect of cinnamon in vitro and in vivo that it decreased MDA levels and increased antioxidant enzymes activities. In another study (Barakat *et al*., 2022), recommended using fennel seeds in foods because of their bioactive health functions and protective effects that may help prevent diseases associated with oxidative stress. Anise extracts exhibit antioxidant and antimicrobial powers which make them effective for treating infectious diseases and fighting free radicals. (Chandra *et al*., 2022). Results of (Mushtaq *et al*., 2019) indicated that anise aqueous extract improves memory by preventing oxidative stress by providing neuroprotection of cholinergic pathways against acetylcholinesterase (AChE). It decreased levels of AChE and MDA in brain homogenates and improved levels of CAT, GSH, and SOD.

Table 7. Serum antioxidant enzymes activity of experimental rats fed on biscuits supplemented with cinnamon, anise and fennel powders.

Variable Groups	SOD (U/ml)	CAT (U/L)	GSH (mmol/L)	MDA (nmol/ml)
G1 (-ve) young control	126.00^{a} ±5.57	1.77^{a} ±0.07	2.57^{a} ±0.04	8.30^{d} ±0.46
G2 (+ve) elder control	54.67^{de} ±6.03	0.36^{e} ±0.03	1.22^{d} ±0.13	25.73^{a} ±1.25
G3: Wheat Biscuits	45.00^{e} ±6.56	0.33^{e} ±0.02	1.07^{d} ±0.10	27.73^{a} ±1.55
G4:Cinnamon biscuit	114.00^{ab} ±8.00	1.35^{b} ±0.21	2.34^{ab} ±0.17d	10.83^{cd} ±1.86
G5: Fennel biscuit	100.67^{b} ±10.60	1.07^{c} ±0.22	2.18^{b} ±0.10	13.80^{c} ±1.97
G6: Anise biscuit	107.00^{b} ±12.77	1.21^{b} ^{c}±0.23	2.24^{b} ±0.17	12.37^{c} ±2.55

Numbers are mean ± standard deviation of 5 rats in each group. Different superscript letters in the same column indicate mean values differ significantly ($p \leq 0.05$).

SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; MDA: Malondialdehyde.

Serum hormones of aged rats fed on biscuits supplemented with cinnamon, anise and fennel powders.

Data represented in Table (8), cleared that fed on basal diet in +ve elder control group (G2), and wheat biscuit (G3), showed a significant increase at P< 0.05 in Luteinizing hormone (LH)S, Parathormone (PTH) and Testosterone, on contrast, they showed a significant decreases at P< 0.05, in both progesterone and osteocalcin serum compared with –ve young control group .

Groups fed on cinnamon powder biscuit (G4), fennel powder biscuit (G5), and anise powder biscuit (G6), showed a significant decreases (P< 0.05), in each of LH, PTH, and testosterone serum. While showed a significant increase (P< 0.05), in both progesterone, and osteocalcin serum compared to +ve elder control group .

Our findings cleared that, cinnamon, fennel, and anise, had the ability of organizing aged female rats' hormones, compared to +ve elder control group.

(Dou *et al*., 2018) reported that cinnamon has the ability to regulate serum levels of LH and testosterone and to restore the shape of the ovaries caused by the condition of PCOS. (Khosrowpour *et al*., 2022), reported that both fennel and anise improved serum levels of progesterone and other sex hormones. (Helal *et al*., 2019) reported that 1 ml / kg body weight / aniseed for a month, reduced FSH, LH, and testosterone. (Wu *et al*., 2018) showed that combined treatment with cinnamaldehyde and PTH enhances osteoblasts in a rat model of osteoporosis.

(Ibrahim, 2017), reported that cinnamon extract helps increase sex hormones, including testosterone and improves testosterone level. (Azam *et al*., 2017) found that administering different concentrations of fennel seeds significantly increased the levels of estrogen and progesterone hormones in the blood of adult female rats. Stated on (Sadeghpour *et al*., 2015) fennel seed extract can increase the level of estrogen and progesterone in the blood as a fertility-promoting agent (Hassan and Saed, 2011) cleared that anise supplementation increases the level of bone formation (alkaline phosphatase and osteocalcin) so it can protect from osteoporosis in rats

Table 8. Serum Hormones of experimental rats fed on biscuits supplemented with cinnamon, anise and fennel powders.

Variable / Groups	LH (mIU/ml)	PTH (pg/ml)	Testosterone (ng/ml)	Progesterone (ng/ml)	Osteocalcin (ng/ml)
G1 (-ve) young control	e 0.21±0.01	e 8.87±0.55	f 0.13±0.02	a 41.10±1.a61	a 32.17±1.43
G2 (+ve) elder control	ab 0.34±0.01	ab 15.20±0.56	ab 0.27±0.01	de 25.60±0.62	de 19.67±0.96
G3: Wheat Biscuits	a 0.35±0.01	a 15.97±0.59	a 0.29±0.02	e 24.43±1.12	e 18.23±0.85
G4: Cinnamon biscuit	de 0.23±0.03	d 10.33±0.84	e 0.20±0.02	b 36.20±1.90	b 27.53±1.99
G5: Fennel biscuit	d 0.26±0.03	d 11.13±0.72	de 0.22±0.02	b 33.60±2.07	b 25.50±1.35
G6: Anise biscuit	d 0.25±0.03	d 10.97±0.97	e 0.21±0.02	b 35.17±2.71	b 26.67±1.91

Numbers are mean ± standard deviation of 5 rats in each group. Different superscript letters in the same column indicate mean values differ significantly ($p \leq 0.05$).
LH: Luteinizing Hormone; PTH: Parathyroid hormone.

CONCLUSION

In conclusion, feeding female aged rats biscuits supplemented with cinnamon, fennel and anise powder may significantly improve the vital functions of the female body such as lipids, liver, kidneys and aging female hormones and enhance the antioxidant defense status, So this study recommends that the use of cinnamon, fennel and anise powder for the elderly, could be of value and safe.

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تأثير البسكويت المدعم بمسحوق القرفة والشمر واليانسون على الوظائف الحيوية في الفئران المسنة

رشا محمد نجيب ، سارة صلاح السيد و مني ياسر عبدالخالق مصطفي

قسم الاقتصاد المنزلي – كلية التربية النوعيه – جامعه المنصورة - مصر

الملخص

يهدف هذا البحث الى دراسة تأثير البسكويت المدعم ب ٥٪ من مسحوق القرفة والشمر واليانسون على الوظائف الحيوية فى ٣٠ من إناث الفئران المسنة ، حيث قسمت إناث الفئران إلى ٦ مجموعات (٥ فئران فى المجموعة) على النحو التالي ، المجموعة (١) المجموعة الضابطة السالبة (الصغيرة) تتغذى على النظام الغذائي الأساسي ، المجموعة (٢) المجموعة الضابطة الموجبة (المسنة) تتغذى على النظام الغذائي الأساسي ، المجموعة (٣) تتغذى على بسكويت القمح والمجموعات ٤ و ٥ و ٦ تم تغذيتها على بسكويت القرفة والشمر واليانسون خلال فترة التجربة (٨ أسابيع). أشارت النتائج إلى أن المجموعات التي تتغذى على البسكويت المضاف إليه بودرة القرفة والشمر واليانسون أظهرت زيادة ملحوظة فى HDL-c و T.Bil و SOD و CAT و GSH والكالسيوم وهرمونات البروجسترون والأوستوكالسين ، بينما أظهرت انخفاض كل من TG، TC، LDL-c، VLDL-c، AST، ALT، ALP ، الكرياتينين ، اليوريا ، حمض اليوريك ، MDA، وهرمونات LH، PTH ، التستوستيرون، مقارنة بمجموعة التحكم الإيجابية المسنة. لذا توصي الدراسة بتناول المنتجات التى تحتوي على مسحوق القرفة والشمر واليانسون؛ لقدرتها على تحسين مستوى الدهون، وظائف الكبد ،الكلى، والهرمونات الأنثوية بالإضافة لدورها كعوامل مضادة للأكسدة.

الكلمات الدالة: القرفة، الشمر، الينسون، البسكويت، الدهون، الكبد، الكلى، الهرمونات، مضادات الأكسدة.