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Potential Effect of Dietary Flaxseed (Linum Usitatissimum L.) Powder and Extract on aged Menopausal Female Rats

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Abstract: *Flaxseed is rich in bioactive food components, and has therapeutic properties in menopause, which is preventing changes in hormonal status and that of the main reasons for osteoporosis after menopause. The present study aimed to evaluate the effect of Flaxseed on reduction and management of postmenopausal symptoms lipid profile and improve antioxidant status in aging female rats. Seven young adult female Sprague Dawley rats (3 month old, 200± 15g) was acted as young negative control rat group and twenty one aged female Sprague Dawley rats (18- 20 month old, 300 ± 15g) were divided into positive control group fed on basal diet and two groups which treated with flaxseed powder (FP) as 15% powder in basal diet and flaxseed water extract (FE) as 1300mg/kg b.w daily by stomach tube. The experiment period was 60 days. The obtained results revealed that flaxseed contain highest amount of phenolic compounds catechein, gallic,pyrogallol and e-vanillic and flavonoids especially ferulic acid, chlorogenic acid , gallic acid , and traces of 4-hydroxybenzoic acid. The biological study showed that consumption of flaxseed powder or extract showed significant increase in body weight gain (BWG), feed efficiency ratio (FER), serum high density lipoprotein cholesterol, (HDLc), follicle stimulating hormone (FSH), luteinizing hormone (LH) , estradiol (E2),and Progesterone(P4) ($p< 0.01$) compared to (+ve) control rat groups.*
a significant decrease in total cholesterol (TC), triglycerides(TG),low density lipoprotein cholesterol(LDLc) and very low density lipoprotein cholesterol(VLDLc) and testosterone hormone($p< 0.01$ & 0.001) comparing with the (+ve) control rat groups. It concluded that consumption of flaxseed has a best significant treatment of symptoms in aged menopausal female rats

Keywords: *Flaxseed - Phenolic compounds - Antioxidant activities -menopausal– Sex hormones – Lipid profile - Rats*

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I. Introduction

Many physical changes occur during the transition from the reproductive years to menopause for most women (Manson, 2008 and Asali *et al.*, 2010). Menopause enhances the psychological and physiological changes greatly affect the health of women and lead to the loss of ovarian work, followed by a permanent interruption of menstruation (Bruce and Rymer 2009 and Goodman *et al.*, 2011). Most women undergo physiological changes in postmenopausal, and complications occur due to the reduction of estrogen including osteopenia and osteoporosis, hot flashes, sweating, general discomfort, insomnia and vaginal dryness (Triggiani *et al*. 2006., Gibbs *et al*. 2008 and Al-Safi and Polotsky 2015). Twelve months aged or above rats showed an abnormal estrous cycle with lower progesterone and estrogen levels (Moorthy et al., 2005).

Flaxseed (*Linum Usitatissimum L.)* is the older of one cultivated plants, starting in the Tigris and Euphrates rivers nearly 8,000 years ago Kim and Choi, (2005). Flaxseed is rich in omega 3 fatty acid that is responsible for its ability to improvement in blood lipids, reduce bad cholesterol (LDL), triglycerides (TG) and blood pressure. It also fights inflammatory reactions in the body (Vijaimohan *et al*. 2006) and Evelyn 2010). Flaxseed has anticancer effects in breast and prostate cancers and it usage in the diet may help to reduce the risk of CVD, stroke and it help to prevent the development of some hormonal disorders and has anticancer effects in breast (Simopoulos 2002; Amit *et al*. 2010 and Kotlyarova, 2012). Flaxseed used as an anti-oxidant, anti-carcinogenic and helps to alleviate hot flashes and reduce the osteoporosis because lignin has particular interest because of his antioxidant activity and may help protect against certain cancers (Chen *et al.* 1994, Malcolmson *et al.* 2000 and Dew and Williamson 2013). Flaxseed used as drug by doses of 25 to 40 g/day in postmenopausal women suffering hot flushes *(Dodin et al.* 2005). This study objective is to evaluate the contents of phenolic compounds and antioxidant activities in the flaxseed with improving the lipid profile and the level of sex hormones and the treatment of symptoms associated with the human menopause.

II. Materials And Methods

Materials:

Flaxseed (*Linum Usitatissimum L)*: Flaxseed was obtained from Medicinal Seeds and Herbs Company, Cairo, Egypt.

Biochemical kits: All the kits for biochemical analysis of serum lipids total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDLc), low density lipoprotein (LDLc) , very low density lipoprotein (VLDLc), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P4) and testosterone were obtained from Kamiya Biomedical Company, Cairo, Egypt.

Experimental animals:

Twenty-eight female rats, young adult (3 month old, n=7) and aged(18 -20 month old, n=21) female Sprague Dawley rats, weighing about 200± 15g g and 300 ± 15g respectively, at the beginning of the experiments were purchased from the Agricultural Research Center, Giza, Egypt.

Methods

Analytical Methods

1. Some chemical composition of flaxseed

a. Preparation of flaxseed formulations**:**

Flaxseed materials was milled in a mixer to give a powder and kept in dusky stoppered glass bottles in a dry location till use, according to **Russo, (2001)** who reported that seed is best kept in a dry and dark location to reduce oxidation of their contents. Flaxseed was extracted in distilled water in ratio 1:10 seven times for eight hours and then concentrated twice under vacuum at 400 ^{0}C.

b. Analysis of flavonoids:

High-performance liquid chromatography (HPLC) was performed by using an Agilent 1200 chromatograph was selected for detection described by the method (**Goupy *et al.* 1999**).

III. Biological Methods

The rats were divided into four groups Group (1) were young negative control rats (3 months old), but Group 2,3,4 were Postmenopausal rats 18 -20 month old according to **Moorthy et al., (2005).** The female rats were housed in animal cages at12-h light/ dark cycle in controlled temperature room. Rats allowed to be fed on basal diet (BD) which prepared according to **NRC (1995).** It consists of 200g casein, 497g starch, 100g sucrose, 20 g vitamin mixture, 50g mineral mixture, 100g corn oil, 3g dl- methionine and 30g cellulose in kg of diet and water *ad libitum* throughout the period of (one week). After one week adaptation, rats were classified to four groups: Group (1): Fed on (BD) considered young negative control group (-ve). Group (2): fed on (BD) Postmenopausal positive control group (+ve). Group (3): fed on (BD) Postmenopausal consumption of flaxseed powder (FP) 15 g/ kg in basal diet as constituents of fiber. Group (4): fed on (BD) Postmenopausal consumption flaxseed extract (FE) dissolved in distilled water at (1300mg/kg b.w daily) by stomach tube. The food intake was monitored daily and the growth of animals was monitored weekly by measuring body weight. Food efficiency ratio (FER) was determined by **Chapman et al., (1950)**.**and Hsu *et al*. (1978)**. Rats were sacrificed after 60 days under light ether anesthesia. Samples of blood were collected from retro-orbital plexus of veins in the inner canthus of the eye using micro capillary tubs and withdrawn in test tubes. The tubes were left for coagulation the centrifuged at 5000 rpm for 10 min and kept at room temperature for 15 minutes. The separated serum was kept frozen in a refrigerator at – 18^{O}C to obtain serum for further analysis **(Young, 2001).** Serum total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL-c) were figured out reference to **Cohn et al., (1988), Foster and Dumns, (1973)** and **Young, (1995),** respectively. Low density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c) were measured as described by **Friedwald et *al.*, (1972).** Athrogenic index was calculated by CH / HDLc according to **Castelli and Levitar (1977).** Serum follicle stimulating hormone (FSH), estradiol (E2), Luteinizing hormone (LH), progesterone (P4) and testosterone levels were determined by radioimmunoassay according to **Ballester et al. (2004), Schams and Karg, (1970), Wilke and Utley.(1987) van der Molen et al., (1965) and Bee and Kah, (2003), respectively**.

IV. Statistical Analysis

The obtained data were statistically analyzed by SPSS computer software expressed as mean ± SD. Effects of various treatments were measured by one-way (ANOVA) test using Duncan's multiple range tests and $P < 0.05$ was considered statistically significant according to **Snedecor and Cochran (1986).**

V. Results And Discussion

Identified content of the phenolic compounds (ppm) in flaxseed

HPLC is the preferred technique for both separation and quantification of phenolic compounds **(Goupy et al. 1999)**. The HPLC analysis of the phenolic compounds flaxseed were compiled in table 1 and figure 1.showed amount of phenolic compounds with an average from 1460.20 to 4.94 ppm. The highest content were catechein, gallic, pyrogallol and e-vanillic and their amount were 1460.20, 872.69, 657.58 and 408.67 ppm, respectively but the lowest content of phenolic compound in flaxseed were Iso-ferulic, ferulic, p-conmaric and cinnamic and their amount were 4.94, 6.24, 6.56 and 6.62 ppm, respectively. The major flavonoid identified in defatted flaxseed powder are ferulic acid, chlorogenic acid , gallic acid , and traces of 4-hydroxybenzoic acid, these results matched with **Westcott *et al.* (2000); Eliasson *et al.* (2003); Arts *et al.* (2001) and Siger *et al.* (2008).**

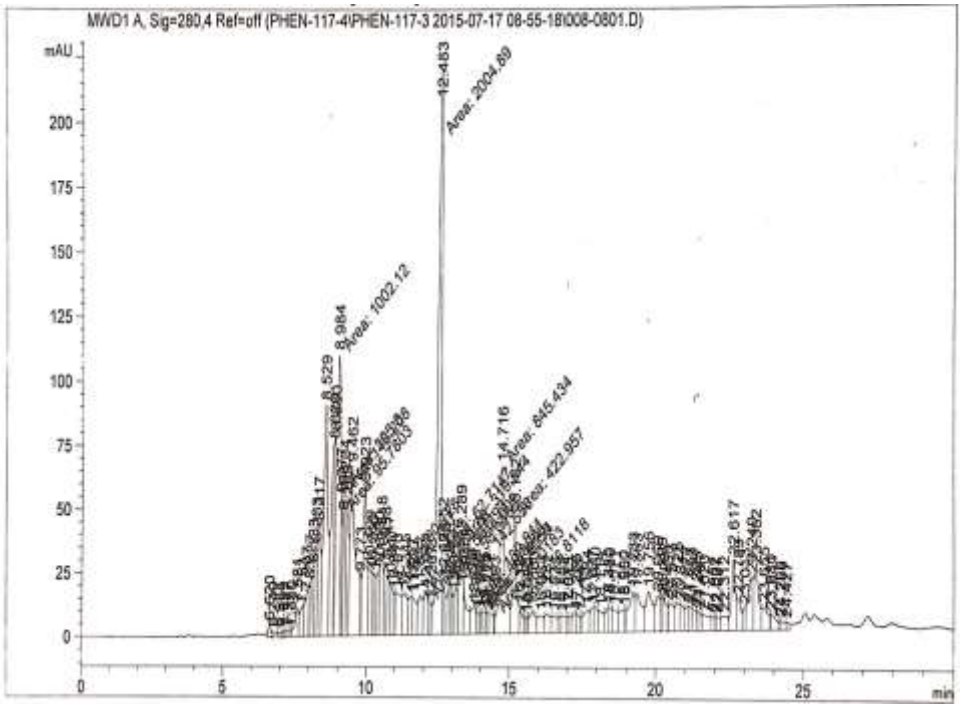

Fig. 1. Total Phenolic compounds content of flaxseed

Table (1) Comparison of Phenolic compounds contents in flaxseed (ppm)

Phenolic compounds	Composition (ppm)	Phenolic compounds	Composition (ppm)
Gallic	872.69	Vanillic	9.62
Pyrogallol	657.58	P-conmaric	6.56
4-Amino-benzoic	53.04	Ferulic	6.24
3-OH-Tyrosol	10.18	Iso-ferulic	4.94
Protocatchuic	61.00	Reversetol	7.87
Chlorogenic	47.81	Ellagic	70.10
Epi-Catachin	--	e-vanillic	408.67
Catechein	1460.20	Alpha -coumaric	19.89
Catechol	18.45	Benzoic	83.96
Caffeine	8.58	3,4,5-methoxy-cinnamic	13.70
P-Oh-benzoic	74.05	Coumarin	7.47
Caffeic	41.45	Salycilic	167.22
Cinnamic	6.62		

Data advanced in table (2) showed that total antioxidant activities (%) of flaxseed recorded (45.47%). Results indicated a positive correlation between the total antioxidant and their phenolic and flavonoid contents. Phenolic compounds of antioxidant have ability to scavenge free radicals to possess antioxidant activity in flaxseed justified by **Prasad, (2000), Niemeyer and Metzler (2003) and Mazandarani, *et al.* (2012).**

Table (2): Antioxidant activities of flaxseed:

Samples code	Total antioxidant activity %
Flaxseed	**45.47**

Data in table (3) showed that the positive control group which postmenopausal rats had a significant lower in weight gain, weight gain and food efficiency ratio (FER) at P< 0.01 in comparison with negative control Young rat group. The postmenopausal rat groups which administered flaxseed powder and flaxseed water extract showed a significant increase (P< 0.001) in weight gain and food efficiency ratio (FER) in comparison with positive control group. While the no significant different in food intake in all postmenopausal rats rat groups compared with negative control group. Our results are in harmony with those obtained by **Felmlee, (2009), Elbostany, et al. (2013), Moghaddam, et al. (2016) and Yari ,et al. (2016)**

Table (3): Body weight gain (g), food intake (g) and feed efficiency ratio (FER) of the experimental rat groups

Groups / Variables	Young rat (-ve) Control	Aged menopausal female rats groups		
		(+ve) Control	Treated with FP(15g/BD)	Treated with FE(1300mg/bw)
Body weight gain (g)	115.77 ±8.11 a	66.89 ± 6.11b	103.14 ±9.13a	103.14 ± 9.13a
Food intake (g/w)	15.32 ± 2.14 a	14.55 ± 2.55a	15.45 ±2.42a	15.45 ±2.42a
FER	0.125 ±0.01a	0.062 ±0.03b	0.111 ±0.02a	0.111 ± 0.02a

Mean values in each row having different superscript are significantly different at (P< 0.05)
FP: Flaxseed powder, FE: flaxseed water

Table (4) showed that the positive control postmenopausal rat group had a significant increase (P< 0.01&0.001) in total cholesterol (TC), Triglyceride (TG),low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc)and atherogenic indexes (TC/ HDLc) but showed a significant decrease (P<0.01) in high density lipoprotein cholesterol (HDLc) compared with negative control Young rat group. Postmenopausal rats which administered flaxseed powder and flaxseed water extract showed a significant decrease (P<0.01) in total cholesterol, Triglyceride, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and atherogenic indexes (TC/ HDLc) wilh a significant increase (P<0.001) in high density lipoprotein cholesterol compared with positive control postmenopausal rat group. Studies have showed an increased risk of heart disease after menopause as reported by **Polotsky and Polotsky (2010), Jouyandeh et al., (2013), Ursoniu et al.,(2016)** and **Ricklefs-Johnson et al., (2017).** Studies of cardiovascular disease showed that a relationship between the increase in consumption of anti-oxidants such as phenolic (e-vanillic, gallic and catechein) and a reduced risk of cardiovascular disease, This agreed with the reports of **Cui et al., (2004); Dodin et al. (2005), Valko et al., (2007)** and **Hassan and Abdel-Wahhab (2012).**

Table 4: Effect of seeds flaxseed powder and extract on the serum lipid pattern and in young and aged menopausal female rat groups at the end of study

Groups / Variables	Young rat (-ve) Control	*Aged menopausal female rats groups*		
		(+ve) Control	*Treated* with FP(15 %BD)	*Treated* with FE(1300mg/bw)
TC(mg/dl)	50.06 ±1.28e	55.57 ±1.03a	52.54 ±0.29bc	51.78 ±0.86b
TG(mg/dl)	52.21 ±1.68b	63.49 ±1.03a	51.18 ±0.78c	52.70 ±0.44b
HDLc (mg/dl)	15.56 ±1.12a	12.02 ±0.66d	14.24 ±0.78b	13.65 ±0.33bc
LDLc(mg/dl)	22.06 ±1.12e	32.85 ±0.79a	28.07 ±1.06d	27.59 ±0.28b
VLDLc(mg/dl)	10.70 ±0.33b	12.44 ±0.22a	10.23 ±0.16c	10.54 ±0.09b
TC/ HDLc)	3.21 ±0.42b	4.55 ±0.16a	3.68 ±0.26b	3.79 ±0.32b

Mean values in each row having different superscript (a, b, c, d and) are significantly different at (P< 0.05, 0.01&0.001)

FP: Flaxseed powder, FE: flaxseed water extract , TC: total cholesterol, TG: Triglyceride, HDLc: high density lipoprotein cholesterol, LDLc: low density lipoprotein cholesterol, VLDLc: very low density lipoprotein cholesterol., atherogenic index TC/ HDLc

The above data in table (5) showed that the positive control postmenopausal rat group showed a significant decrease (P< 0.01&0.001) in Serum follicle stimulating hormone (FSH), luteinizing hormone (LH)), estradiol (E2), Progesterone (P4) but significant increase at P<0.001 in serum testosterone hormone compared with negative control Young rat group. Postmenopausal rats which administered flaxseed powder and flaxseed water extract showed a significant elevation at P<0.001 in serum follicle stimulating hormone (FSH), luteinizing hormone (LH)), estradiol.(E2), Progesterone (P4) but significant decrease at P<0.001in serum testosterone hormone in comparison with positive control postmenopausal rat group .The highest mean values in FSH, LH, E2 and P4 found in groups administered flaxseed powder and flaxseed water extract that the due to flaxseed reduce the risk of tumors caused by estrogen deficiency, these results were similar to **Moghaddam, et al. (2016).** Flaxseed is a rich source of phytoestrogens, studies have proved good its effect on the sex hormones observed by **Hutchins et al. (2001).** Flaxseed and flaxseed oil may help to prevent osteoporosis resulting from estrogen deficiency suggested by **Hala et al (2011**). The behavior health of the flaxseed lignans depends on the levels of estradiol, the lignans has estrogen antagonist effect, but in postmenopausal women at low estradiol levels and weak estrogens **Hutchins and Slavin (2003)**

Table 5: Serum follicle stimulating hormone (FSH), luteinizing hormone (LH)), estradiol (E2), Progesterone (P4) and testosterone hormone of the experimental rats groups

Groups / Variables	Young rat (-ve) Control	*Aged menopausal female rat groups*		
		(+ve) Control	*Treated* with FP(15g/BD)	*Treated* with FE(1300mg/bw)
FSH(mg/dl)	5.04±0.16a	3.62±0.07e	4.48±0.10b	4.17±0.53c
LH(mg/dl)	3.21±0.25a	2.33±0.07f	2.82±0.09c	2.67±0.04c
E2 (mg/dl)	23.05±0.80a	11.74±0.79f	20.76±0.85c	19.72±0.89c
Progesterone (mg/dl)	0.88±0.06a	0.27±0.02f	0.69±0.05c	0.58±0.04cd
Testosterone (mg/dl)	0.11±0.02e	0.27±0.04a	0.16±0.02c	0.18±0.01c

Mean values in each row having different superscript (a, b, c, d and) are significantly different at (P< 0.05, 0.01&0.001) .FP: Flaxseed powder, FE: Flaxseed water extract,

VI. Conclusion

In conclusion, the biochemical results reported in the current study confirmed and indicated that increased consumption of flaxseed powder and extract lowered LDL-c, total cholesterol levels thus decreasing the risk of cardiovascular disease, while increase in HDL-c and improving the level of sex hormones. These effects of flaxseed powder and extract might be a result of their high content of polyphenols and other antioxidants like flavonoids that could scavenge the free radicals of postmenopausal in woman. It is recommended to administer of flaxseed powder and extract for menopausal woman to improve morphological changes in postmenopausal.

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