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Effect of Wheat Germ, Wheat Germ Oil on Lipid Profile of Hypercholesterolemic Rats

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Abstract

Hypercholesterolemia plays an important role in atherosclerosis and cardiovascular diseases (CVD) that represent one of the greatest worldwide medical problems nowadays. Recently, an increased attention/ interest for natural antioxidant/ hypercholesterolemia as WG/WGO is increasing. Wheat germ/ Wheat germ oil (WG/WGO) is an excellent source of essential and polyunsaturated fatty acids and vitamin E. It is one of the richest natural sources of tocopherol. This work aims to evaluate the effect of administration of wheat germ (WG) /wheat germ oil (WGO) for 6 weeks on serum lipid profile, lipid peroxidation (liver malondialdehyde) in hypercholesterolemic rats. One hundred and twenty male Sprague Dawely rats weighing 180-192 gm were used in this study. They were randomly distributed into six groups (20 rats/ group) as follow: g1: normal control, G2: hypercholesterolemic rats; G3: Normal rats treated with WG; G4: hypercholesterolemic rats treated with WG; G5: Normal rats treated with WGO; G6: hypercholesterolemic rats treated with WGO for 6 weeks. Also WG was analysed for its nutritive value, while WGO was analysed for fatty acid profile, and studied for its physico-chemical properties. The results showed significant elevation of liver malondialdehyde (MDA) and serum lipid profile in untreated rats fed on hypercholesterolemic diet. Hypercholesterolemic rats treated with WG/WGO showed an improvement in the biochemical assay of their lipid profile compared with untreated hypercholesterolemic rats. WG/WGO may be able to protect against atherosclerosis and CVD. The administration of WG /WGO to diets of rats caused a marked reduction in TC, LDL-C, VLDL-C, TG and MDA. This improvement effect may be mediated via enhancement of the antioxidant defence system and other factors. However, further clinical studies on human beings are required to assess the efficacy and safety of the WG/ WGO.

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Introduction

Body can get cholesterol through diet (exogenous) or synthesis (endogenous). Dietary cholesterol can affect lipid profile in human and animals. Feeding cholesterol-rich diets leads to hypercholesterolemia. Cholesterol homeostasis is controlled through absorption, synthesis and excretion. Hypercholesterolemia plays an important role in heart damage, stroke and atherosclerosis (1] and associated with cardiovascular diseases (CVD) that represent one of the greatest worldwide medical problems nowadays [2].

The National Cholesterol Education program [3] and [4], both allow consuming specific food component as plant sterol and dietary fibre that can lower cholesterol, LDL-C so we choose WG/WGO.

Wheat germ is a by-product of the flour milling industry and is an excellent source of vitamins, minerals, fibre and proteins [5]. Also wheat germ is one of the richest known natural sources of tocopherols. Wheat germ protein is rich in amino acids, especially the essential amino acids lysine, methionine and threonine, in which many cereals are deficient [6].

Wheat germ oil (WGO) is an excellent source of essential polyunsaturated fatty acids and vitamin E [7]. It is one of the richest natural sources of tocopherol (α, β, g), and α & g tocotrienols. Also it contains phytosterols, mainly camp sterol, β-cytosterol, which have strong antioxidant activity [8], and [7], carotenoids and phenolic compounds [9].

Wheat germ oil might reduce plasma and liver cholesterol in animals, [10], reduce oxidative stress and improve lipid metabolism [11]; and [12].

Therefore the aim of the present study was to evaluate the effect of administration of wheat germ (WG) /wheat germ oil (WGO) for 6 weeks on serum lipid profile, lipid peroxidation (liver malondialdehyde) in hypercholesterolemic rats. Also would WG / WGO provide protection against oxidative stress in rats?

Materials and Methods

Experimental Animals

One hundred and twenty (120) male albino rats, average body weight 180-192 gm were used in the experiment. Rats were housed in separate wire mesh cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The diet and water were ad libitum. The animals were left 10 days for acclimatization before the beginning of the experiment. The standard rat diet (AIN-93 M diet) was prepared according to [13] [14]. Hypercholestrolemic diet was prepared by supplementation of the basal control diet with cholesterol (1%), and cholic acid (0.5%) [15].

Experimental Design

After acclimatization period, 120 adult male Sprague Dawely rats were divided into 6 groups, (20/ group) as follow:

Group 1: Normal, - ve control group, rats were fed on control diet.

Group 2: + ve control group, rats were fed on hypercholesterolemic diet.

Group 3: Normal rats fed on control diet supplemented with wheat germ (30%).

Group 4: Hypercholesterolemic rats treated with wheat germ (30%).

Group 5: Normal rats fed on control diet supplemented with wheat germ oil (1 g/kg BWt).

Group 6: Hypercholesterolemic rats treated with wheat germ oil (1 g/kg BWt).

After the end of the experiment (6 weeks), rats were fasted overnight, and then scarified under ether anaesthesia (Sigma, USA). Blood samples were taken by cardiac puncture. Fasting blood samples were collected in plain tubes, centrifuged for separation of serum at 3,000 rpm for 15 minutes, and sera were stored at –20 ° C for determination of the following biochemical measurements: total cholesterol, HDL-C, LDL-C, VLDL-C, triacylglycerol. Rat livers were excised to be used for determination of malondialdehyde after being washed with saline, dried and weighed. They were kept at -20 ° C till analysis.

Total cholesterol, TC; Serum HDL-C; and Serum LDL-C; was determined using SGM Italia, Rome, kit (Italy) according to the method of Allian *et al.,* [16]; Lopes-Virella *et al.,* [17]; Fruchart *et al.,* [18] and Levy *et al.,*[19]; and Bucolo and David [20] respectively. VLDL-C was determined by using the following equation: VLDL-C=total cholesterol- (HDL-C+LDL-C). Atherogenic Index (AI) was calculated according to Lee and Niemann

[21] using following equation: AI= (Total cholesterol-HDL-C)/HDL-C. Malondialdehyde was determined according to the method of Yoshioka *et al.,* [22] and [23].

Rats were weighed twice/week to calculate Body weight gain (BWG) using the equation: BWG = Final body weight – initial body weight.

Wheat Germ/ wheat germ oil

Wheat germ was analysed for its proximate composition: moisture, ash, fat, fibre, dietary fibre and protein according to AOAC [24] standard methods. Fatty acid composition were determined accordance to the method of Morrison and Smith [25] Santos *et al.,* [26], ISO 12966-2 [27] and ISO 15304 [28]. Also chemical characteristics of wheat germ oils were assessed in terms of acid value, iodine value, saponification value, peroxide value, ester value and unsaponifiable matter. They were determined according to standard IUPAC methods for the analysis of oils and fats [29]. Determination of phytosterol content was done according to Laakso [30]. The content of minerals and vitamins was assayed by means of the AAS flame technique,using a Unicam 929 apparatus (AA Spectrometer Unicam) and HPLC respectively using standard methods of AOAC [24] .

Statistical Analysis

Data are expressed as Mean ±SEM. All statistical data and significance tests (T Test for comparison between individual groups and control group; and post hoc Duncan test analysis for comparison between groups) were performed using the Statistical Package for the Social Sciences version 11 (SPSS Inc, Chicago, IL, USA). Statistical significance was accepted at $P < 0.05$

Results

Results of table 1 a,b,c,d illustrate nutritional value of WG. The results showed that WG contains high levels of protein (23.0%), Fat, crude fibres, and ash contents were 10.0, 1.32 and 4.21 %, respectively (table 1a). WG has high dietary fibre content 16.0. Wheat germ contains 21 mg of vitamin E per 100g (210 mg/100 g oil). Wheat germ oil provides 20 mg vitamin E/ tbsp i.e. more than the recommended daily allowance of 15 mg of vitamin E (table 1b).The most abundant minerals (mg/100g) were: Potassium (1050.0) and phosphorus (999.0) (table 1c). Moreover, wheat germ showed to be a good source for essential amino acids. Saponification, iodine, peroxide values and unsaponifiable matters of the WGO were 187.25, 114.21, 3.5 and 4.33 respectively (table 1d).

Results of table 2 illustrate lipid profile of WGO. The fatty acid profile was found to be made up of linoleic, ω-6 (53.21%) followed by palmitic (16.7) and oleic (15.36) as the major fatty acids. The free fatty acids were 9.64%. The wheat germ oil was found to be an incredible source of ω 3 (7.92) and ω 6 (53.21) essential fatty acids. Furthermore, MUFAs and the unsaturated fatty acids represent around 17.86 % and 79.07% of the total fatty acids respectively.

Results of table 3 illustrate Initial, Final and Body weight gain of the treated rats. Initial body weights (IBW) were comparable between all studied groups. However, final body weight (FBW) of all treated groups became significantly ($P<0.001$) lower than normal controls and significantly higher than untreated group ($P<0.001$, Table 3) being

Results of table 4 revealed that hypercholesterolemic rats (g 2), showed significant increases in serum levels of TC, TGs, LDL-C, VLDL-C, AI and significant decreases in HDL-C when compared to the other groups. Liver MDA level was the highest in hypercholesterolemic rats. The oral administration of the WG/WGO to rats in experimental groups (3-6) significantly decreased serum lipid profile (TC, TGs, LDL-C, and VLDL-C), AI, liver MDA and significantly increased HDL-C level when compared to control negative group.

Discussion

The nutritive value and physicochemical properties of WG agree with Sakhawat *et al.,* [31].

Analysis of wheat germ oil showed that WGO is a good source of Linolenic, ω-3 (7.92) and Linoleic, ω-6 (53.21) and the ω-6/ ω-3 ratio of WGO was 6.7. It has been also suggested that ω-6/ ω-3 ratio of 10 or less results in a reduction in fatal CHD risk [32]; and [33]. Wheat germ oil also has an excellent ω-6/ω-3 fatty acid ratio. According to Ribarova *et al.,* [34] polyunsaturated fatty acids must make up 10% of the total energy ingested for an adequate diet. The consumption of

Table 1a. Chemical composition of WG

Protein	23.0
Fat	10.0
Dietary fibre	16.0
Ash	4.21
Moisture	11.12
CHO	50.35
Fibre	1.32

Table 1b. Vitamin content of wheat germ.

Folate	270.0 mg
Folic acid	0.00 mg
Niacin	5.2 mg
Pantothenic acid	1.91 mg
Riboflavin B2	0.71 mg
Thiamin B1	1.52 mg
Vitamin A	0.00 IU
B 11	82.0 mg
Vitamin B12	0.00 mg
Vitamin B6	1.300 mg
Vitamin C	0.0 mg
Vitamin D	0.00 IU
Vit E(tocopherols)	21.0 mg

Table 1c: Mineral content of wheat germ.

	mg
Calcium, Ca	40.5mg
Copper, Cu	0.91mg
Iron, Fe	5.9 mg
Magnesium, mg	250 mg
Manganese, Mn	15.1mg
Phosphorus, P	990.0mg
Potassium, K	1050.0mg
Selenium, Se	79.0 mg
Sodium, Na	8.0 mg
Zinc, Zn	14.7mg
Calcium, Ca	40.5mg
Copper, Cu	0.91mg
Iron, Fe	5.9 mg

Table 1d. Physicochemical characteristics of WGO.

Acid value (mg/g oil)	0.6
Acidity (as %oleic acid)	0.83
Saponification value (mg/goil)	187.25
Ester value (mg/g oil)	186.65
Iodine value (g I_2/100 g of oil)	114.21
Peroxide value (meqO_2/kg oil)	3.5
Unsaponifable matter (%)	4.33

Table 2. Fatty acid profile of WGO/WG

Fatty acids		WGO %
Lauric	C 12:0	0.02
myristic	C 14:0	0.12
pentadecanoic	C 15:0	0.15
Palmitic	C16:0	16.7
Palmitoleic	C16:1	0.24
Margaric acid	C 17:0	0.04
Stearic	C18:0	2.06
eliadic	C18:1n-9t	0.8
Oleic	C18:1	15.36
Linoleic (ω-6)	C18:2	53.21
Linolenic (ω-3)	C18:3	3.5
linolenic	C18:3n-3	4.42
Arachidic	C20:0	0.25
Gadoleic	C20:1	1.21
11-Eicosenoic acid (ω-9)	C20:1	0.08
heneicosylic	C21	1.3
Behenic	C22:0	0.29
Total saturated		20.93
Total unsaturated		79.07
∑MUFA		17.86
∑PUFA		61.21
ω-6		53.21
ω-3		7.92
ω-6/ω-3		6.72
pufa/sfa		2.92
sfa/pufa		0.34
oleic/linoleic		0.29
S/U		0.26: 1
U/S		3.78: 1

Table 3. Initial, Final and Body weight gain of the treated rats

	-ve Cont	+ve Cont	Norm & WG	Cholest & WG	Norm & WG	Cholest& WGO
	G 1	G 2	G 3	G 4	G 5	G 6
ZeroTime	184.70±0.70[a]	184.95±0.72[a]	183.95±0.70[a]	184.65±0.77[a]	183.70±0.45[a]	183.65±0.48[a]
End	261.70±2.26[a]	221.85±0.85[b]	255.40±0.87[c]	247.05±0.64[d]	250.90±0.92[e]	239.20±0.92[f]
BWG	77.00±2.25[a]	36.90±1.01[b]	71.45±1.18[c]	62.40±0.86[d]	67.20±1.00[e]	55.55±1.24[f]

Table 4. Cholesterol, HDL-C, LDL-C, VLDL-C, TG, Liver MDA concentration in treated experimental rats and their Athrogenic index.

	-ve Cont	+ve Cont	Norm &WG	Cholest&WG	Norm &WGO	Cholest& WGO
	G 1	G 2	G 3	G 4	G 5	G 6
Cholesterol	94.7±0.70 [a]	164.95±0.72[b]	87.55±1.20[a, c]	119.65±0.77[d]	89.30±0.97 [c,e]	128.65±0.48[f]
HDL-C	44.08±0.25 [a]	39.65±0.09 [b]	43.72±0.11[a]	42.45±0.07[c]	42.83±0.11 [d]	41.58±0.10[e]
LDL-C	32.55±0.88 [a]	89.68±0.66 [b]	23.76±1.31 [c]	56.45±0.96[d]	25.95±0.67 [c,e]	63.75±0.50[f]
VLDL-C	18.07±0.28 [a]	35.62±0.34 [b]	20.07±0.28 [c]	20.75±0.42[c,d]	21.27±0.38 [d,e]	23.32±0.38[f]
TG	90.35±1.39 [a]	181.64±1.75[b]	81.35±1.39 [c]	105.82±2.13 [d]	85.98±1.53 [e]	118.94±1.93[f]
Liver MDA	14.87±0.24 [a]	22.70±0.22 [b]	15.03±0.14 [a,]	20.07±0.28 [c]	16.57±0.14 [d]	21.39±0.17 [e]
AI	1.15±0.02 [a]	3.16±0.02 [b]	1.00±0.03 [c]	1.82±0.02 [d]	1.09±0.02 [e]	2.09±0.01 [f]
TG/HDL-C	2.05±0.03 [a]	4.58±0.05 [b]	1.86±0.03 [c]	2.49±0.05 [d]	2.01±0.04 [d]	2.86±0.05 [e]

18:2n-6 (linoleic acid) is commonly thought to be capable of reducing LDL and total cholesterol. Several scientific studies have shown that ω-3 fatty acids have benefits for lowering CHD risk. The SFAs% was 20.93%. The ratio of saturated fatty acids to unsaturated fatty acids (S: U), a commonly used criteria to describe the nutritional value of fat, was low for wheat germ oil (0.26).

Wheat germ reduction of cholesterol, LDL-C, VLDL-C and TG may be due to presence of some proteins with pancreatic lipase inhibiting properties; interfere with lipid hydrolysis in the stomach and small intestine, thus inducing a decrease in the intestinal absorption of lipids and dietary cholesterol [35] [36]; or due to presence of policosanol in WG/WGO which inhibit the absorption of bile acids [37] [38], decreased cholesterol synthesis [11], reduce LDL-C level and enhanced cholesterol catabolism [39], or may be due to apo-E receptors responsible for hepatic uptake of chylomicron remnants [40] where chylomicrons are totally taken up by the liver leading to its accumulation in the liver and lower intestinal absorption [41]; Also presence of high concentration of vitamin E, powerful antioxidant, which can inhibit the oxidative modification of LDL-C [42].

Wheat germ has a high content of biologically active phytosterol (340.7 mg %) that might have a role in reducing cholesterol absorption leading to lower serum cholesterol level and LDL-C [43] [44].

Wheat germ oils are rich in linolenic acid which aid in a higher cholesterol secretion into the bile and depletion of the intra-hepatic pool of cholesterol, leading to an increase in cholesterol synthesis and turnover [45]. Also wheat germ oil might reduce hepatic lipid accumulation by stimulating β-oxidation and suppressing fatty acid synthesis [46].

Essential fatty acids, ω 3, of WGO exert antioxidant effect as by inhibiting certain enzymes, which mediate the generation of free radicals, thus reducing the amount of free radicals generated [7]. The anti-atherosclerotic effect WGO may be also exerted via inhibition of oxidative stress mediated CD40 ligand up regulation [47].

WG/ WGO reduction of triacylglycerol may be due to presence of monounsaturated fatty acid, vitamin E and phytosterol. Also may be due to an increase in membrane permeability and fluidity [48] or due to the inhibition of pancreatic lipase and the reduction in triacylglycerol lipolysis [35].

Wheat germ reduction of cholesterol, LDL-C, VLDL-C and TG may be due to high dietary fibre content (16.0%) which might have some effects on cholesterol metabolism. The consumption of dietary fibre has been shown to be inversely associated with CHD [49]. Streppel *et al.,* [50] stated that each intake of 10g/ day of Dietary fibre in the diet might reduce CHD mortality by 17%.

The WG/ WGO induce the tocopherol-mediated redox system and inhibit the synthesis of eicosanoid, which activates the lipid peroxidation (LPO) process [51]. The WGO also contains fat-soluble carotenoids such as lutein, zeaxanthin, beta-carotene tocopherols, flavonoids and phenolic acids, which have antioxidant effect [12];[52].

Hypercholesterolemia induces oxidative stress resulting in increased risk for atherosclerosis development [53] by causing a reduction in the enzymatic antioxidant defence potential of tissues, generation of free radicals and an imbalance between free radicals production and antioxidant levels leading to elevated oxidative stress and accelerated lipid peroxidation, atherosclerosis and heart diseases [54].

The antiatherogenic effect of WGO was attributed to tocopherols, phytosterols, phenolic compounds and unsaturated fatty acids. Tocopherols considered as strong antioxidant due to its radical scavenging activity preventing cell membranes lipid peroxidation [8]; Also phytosterols which have an antioxidant effect [43]; phenolic compounds which might have free radical scavenging activity and unsaturated fatty acids [9]. The ratio of TG/HDL-C for hypercholesterolemic rats is significantly higher than normal control group (4.58 vs 2.05) indicating an abnormal atherogenic lipid profile [55].

Malondialdehyde is a good indicator of lipid peroxidation and reflects the degree of oxidation in the body. In the current study increase in MDA level was observed in the hypercholesterolemic positive control group as compared to normal negative control group. Also, data showed a significant decrease in total

antioxidant capacity level in the positive control as compared to negative control.

Addition of WG / WGO to diets of rats caused a marked reduction in MDA concentration and an improvement in total antioxidant capacity. Juskiewicz *et al.,* [56] demonstrated that wheat germ contains compounds such as benzoquinones and other plant flavonoids which increase the antioxidant potential of serum and control oxidative stress and cell damage.

Conclusion

WG/WGO may be able to protect against atherosclerosis and CVD. The administration of WG / WGO to diets of rats caused a marked reduction in TC, LDL-C, VLDL-C, TG and MDA. This improvement effect may be mediated via enhancement of the antioxidant defence system and other factors. However, further clinical studies on human beings are required to assess the efficacy and safety of the WG/WGO.

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Conflicts of Interest

There are no conflicts of interest.

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