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Preventive Effect of Wheat Germ on Hypercholesteremic and Atherosclerosis in Rats Fed Cholesterol-Containing Diet

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Abstract: Wheat germ is one of the most potential and excellent sources of vitamins, minerals, fiber and proteins. The aim of the present study was to investigate the preventive effect of wheat germ on hypercholesteremic and atherosclerosis in rats fed cholesterol-containing diet. Five groups of rats were used; group (1) fed cholesterol free-diet (negative group); group (2) fed cholesterol-diet (positive group); groups (3), (4) and (5) were fed cholesterol-diets with adding 5, 10 and 15% wheat germ, respectively. Results revealed that positive control rats had significantly increased in serum levels of Total Lipids (TL), Triglycerides (TG), Total Cholesterol (TC), LDL-C, VLDL-C, GOT, GPT and ALP and significant decrease in serum level HDL-C which represented by increased atherogenic index as compared to the negative control groups. Histopathological examination showed that positive control rats had vaculations of tunica media, narrowing in the lumen, focal necrosis of tunica intima and tunica media associated with leucocytic cells infiltration of aorta. In addition to vaculations of cardiac myocytes associated with intramuscular edema as well as cytoplasmic vacuolization and fatty changes of hepatocytes. Feeding different levels of wheat germ caused significantly decreased in serum levels of TL, TG, TC, LDL-C, VLDL-C, GOT, GPT and ALP and significantly increased in serum level of HDL-C which represented with significantly decreased in atherogenic index as compared to the positive control group. Histopathological examination revealed that aorta, heart and liver sections of rats feeding 10 and 15% wheat germ had normal histological structure, except, some sections of group treated with 10 % wheat germ had small vacuoles in the cytoplasm of hepatocytes.

Key words: Rats, lipid profile, wheat germ, hypercholesteremic

INTRODUCTION

Wheat kernel is one of the most stable foods. It consists of 81-84% endosperm, 14-16% bran and 2-3% germ (Atwell, 2001). Wheat germ, being a by-product of the flour milling industry and is one of the most potential and excellent sources of vitamins, minerals, fiber and proteins at a relative low cost (Nichelatti and Hidvegi, 2002). It contains on 3 times as much protein of high biological value, 7 times as much fat, 50 times as much sugar and 6 times as much mineral as compared to flour content from the endosperm (Atwell, 2001). In addition to, wheat germ is the richest known natural source of tocopherols and also abundant in B-group vitamins (Holland *et al*., 1991), phytosterols, policosanols (Atwell, 2001) and concentrated source of folate (folic acid), phosphorus, zinc and magnesium, as well as essential fatty acids and fatty alcohols (Cohen, 2003). Wheat germ protein has been classed with effectively better animal proteins and is rich in seventeen amino acids, especially the essential amino acids lysine, methionine and threonine, in which many cereals are deficient (Yiqiang *et al*., 2001).

Atherosclerosis is one of the major health problems in developed countries. It is a degenerative process of the vascular wall, resulting in the following cardiovascular disease: ischemic heart disease, atherosclerotic occlusive disease of lower extremities, ischemic cerebrovascular attacks and other organ damage according to the localization of vascular atherosclerotic changes (Horejsi, 2000).

Atherosclerosis results in Coronary Heart Disease (CHD), one of the major causes of morbidity and mortality. The link between elevated cholesterol and CHD has been clearly established and the National Cholesterol Education Program clinical guidelines for the treatment of hypercholesterolemia identify low-density lipoprotein cholesterol as the primary treatment target (Szapary and Rader, 2004).

Hypertriglyceridemia increase the risk of acute coronary events and some clinical trials found high serum triglycerides to be an independent risk factor for cardiovascular disease (Assmann *et al*., 1996; Yarnell *et al*., 2001). The purpose of this study was to investigate the effectiveness of different levels of wheat germ on

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protective of the risk factors of atherosclerosis-i.e., total lipids, triglycerides, total cholesterol, lipoprotein cholesterols as well as the effect on liver functions and histopathological changes in aorta, heart and liver in rats fed cholesterol-containing diet.

MATERIALS AND METHODS

Rats and diet: Male Sprague-Dawley rats weighing 195±3 g were purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Egypt. Cholesterol, bile salts and basal diet constituents were obtained from El-Gomhorya Company for Chemical and Pharmaceutical, Cairo, Egypt.

Wheat germ: Wheat germ was obtained from Cairo North Mill Co., Cairo Egypt.

Chemicals: Kits for biochemical analysis of serum total lipids, triglycerides, total cholesterol, HDL-C, GOT, GPT and ALP were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Preparation of basal and cholesterol containing-diets: The basal diet (AIN-93M) was prepared according to Reeves *et al*. (1993). Diet was formulated to meet the recommended nutrients levels for rats. Cholesterol-containing diet was prepared by formulated basal diet with 1% cholesterol and 0.25% bile salts to induced hypercholesteremic in rats as described by (Cara *et al*., 1991).

Experimental design: Thirty five rats weighing 195±3 were housed in healthy condition at temperature rooms (21-23°C), with 40-60% humidity, exposed to a 12:12-h light-dark cycle and fed on the basal diet and water was provided *ad libitum* for one week before starting the experimental for acclimatization. After acclimatization period rats were divided into five groups of seven rats each as follows:

Group (1): Served as a control negative group (normal rats) and fed on cholesterol free-diet for 6 weeks.
Group (2): Kept as a control positive group and fed on cholesterol containing-diet for 6 weeks.
Group (3): Fed on cholesterol containing-diet with added 5% wheat germ.
Group (4): Fed on cholesterol containing-diet with added 10 % wheat germ.
Group (5): Fed on cholesterol containing-diet with added 15 % wheat germ.

At the end of the experimental period (6 weeks), diets were withheld from experimental rats for 12-h and then rats were sacrificed. Blood samples were collected from the portal vein into dry clean centrifuge tubes for serum separation. Serum samples were frozen at -10°C until chemical analysis. Aorta, heart and liver of sacrificed rats were kept in 10% formalin solution till processed for histopathological examination.

Determination of food intake, body weight gain and feed efficiency ratio: Food Intake (FI) was calculated every other day, The biological value of the different diets was assessed by the determination of its effect on Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) at the end of the experimental period using the following formulas:

$$BWG = \text{Final body weight} - \text{Initial body weight}$$

$$FER = BWG\ (g)/\text{Food consumed}\ (g)$$

Lipid profile and lipoprotein cholesterol assay: Total Lipid (TL) concentrations were determined colorimetric using spectrophotometer apparatus adjust at 520 nm as described by kit instructions (Randox Co., Ireland). Triglycerides (TG), Total Cholesterol (TC) and High Density Lipoprotein Cholesterol (HDL-C) concentrations were determined using enzymatic methods as described in the instructions provided with the kits (Analyticon® Biotechnologies AG, Germany). The absorbance of the testes samples were read using spectrophotometer adjusted at 546 nm for TG, TC and 500 nm for HDL-C.

Low Density Lipoprotein Cholesterol (LDL-C) concentration was calculated by using formula of Friedwald *et al*. (1972) and Very Low Density Lipoprotein Cholesterol (VLDL-C) was calculated using the following equation:

$$\text{LDL-Cholesterol} = \text{Total cholesterol} - (\text{HDL-C} + \text{TG}/5)$$

$$\text{VLDL-C (mg/dL)} = \text{TG}/5$$

Atherogenic index assay: Atherogenic Index (AI) was calculated using the following equations as described by Dobiasova and Frohlich (2001).

$$\text{Log (TG/HDL-C)}$$

Liver functions assay: Serum aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) and Alkaline Phosphatase (ALP) activities were determined using colorimetric methods as described in the kits instruction (Diamond Co, Hannover, Germany). The absorption of the test samples were read at 505nm for GOT and GPT and at 510 nm for ALP.

Histopathological examination: Aorta, heart and liver of the scarified rats were taken and immersed in 10%

formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Heamtoxylin and Eosin stain for examination of the liver as described by Carleton (1979).

Statistical analysis: Data were expressed as mean± standard deviation. In order to compare the groups, Analysis of Variance (ANOVA) was used. P<0.05 values were considered to be statistically significant.

RESULTS

Body weight gain, food intake and feed efficiency ratio: The effect of cholesterol-containing diet and effect of feeding wheat germ on Food Intake (FI), Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) are record in Table 1. Tabulated data show that rats fed cholesterol-diet (positive control rats) had lower mean±SD value of food intake (20.86±0.69 g/d) which was not significantly changes at p<0.05 as compared to the rats fed cholesterol-free diet (21.29±1.12 g/d). Rats fed cholesterol-diets with added 5 and 10% wheat germ had no significant changes in food intake (20.43±1.13 and 19.86±1.22 g/d, respectively) at p<0.05 as compared to the positive control rats (20.86±0.69 g/d). However, adding wheat germ at the higher level (15%) to the cholesterol-diet caused significant decrease in food intake (19.29±0.95 g/d) at p<0.05 as compared to the positive control group (20.86±0.69 g/d).

With regard to the effect of feeding wheat germ on Body Weight Gain (BWG) in hypercholesteremic rats, the present data demonstrated that positive control group had significant decrease in BWG at p<0.05 (48.71±1.38 g) as compared to the negative control rats (53.29±1.38 g). Rats fed cholesterol-diets with added different levels of wheat germ (5, 10 and 15%) had significant increase in BWG at p<0.05 (52.43±1.72, 51.86±1.99 and 51.43±1.86 g, respectively) as compared to the positive control rats (48.71±1.38 g).

Concerning Feed Efficiency Ratio (FER), results revealed that positive control group had lower mean±SD value of FER which was not significantly changes at p<0.05 (2.34±0.17) as compared to the negative control group (2.51±0.17). Added different levels of wheat germ (5, 10 and 15%) on cholesterol-diet induced significant increase in FER p<0.05 (2.57±0.13, 2.63±0.16 and 2.60±0.27, respectively) as compared to the positive control group (2.34±0.17).

Table 1: Body weight gain, food intake and feed efficiency ratio in rats fed cholesterol-diet

	Parameters as Mean±SD		
Groups	FI (g/d)	BWG (g)	FER
Negative group	21.29±1.12^{a}	53.29±1.38^{a}	2.51±0.17^{ab}
Positive group	20.86±0.69^{ab}	48.71±1.38^{b}	2.34±0.17^{b}
Wheat germ (5%)	20.43±1.13^{abc}	52.43±1.72^{a}	2.57±0.13^{a}
Wheat germ (10%)	19.86±1.22^{bc}	51.86±1.99^{a}	2.63±0.16^{a}
Wheat germ (15%)	19.29±0.95^{c}	51.43±1.86^{a}	2.60±0.27^{a}

Means in each column with different superscript letters differ significantly at p<0.05. A uses harmonic mean sample size = 7 rats

Lipid profile: Data in Table 2 demonstrated that rats fed cholesterol-diet (positive control group) had significant increases (p<0.05) in serum concentrations of Total Lipids (TL), Triglycerides (TG) and Total Cholesterol (TC) (391.86±4.10, 60.14±2.12 and 106.00±2.71 mg/dL, respectively) as compared to the negative control group (315.43±3.15, 36.29±3.30 and 73.26±1.70 mg/dL, respectively). Rats fed cholesterol-diets containing different levels of wheat germ (5, 10 and 15%) had significant decreases (p<0.05) in serum concentrations of TL, TG and TG as compared to the positive control rats. Treated groups with 15% wheat germ had the lower mean±SD values in serum concentrations of TL, TG and TC which were significant decreases at p<0.05 as compared to the other treated groups with 5 and 10% of wheat germ.

Lipoprotein cholesterols and atherogenic index: Results in Table 3 revealed that positive control group had significant increase in serum level of LDL-C at p<0.05 (72.43±3.91 mg/dL) as compared to the negative control group (37.89±3.14 mg/dL). Administration of different levels (5, 10 and 15%) of wheat germ caused significant reduction in serum level of LDL-C at p<0.05 (58.34±2.42, 43.57±4.27 and 36.86±2.57 mg/dL, respectively) as compared to the positive control group (72.43±3.91 mg/dL).

Table 2: Serum concentrations of total lipid, triglycerides and total cholesterol in rats fed cholesterol-diet

	Parameters as Mean±SD		
Groups	Total lipid (mg/dL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)
Negative group	315.43±3.15^{d}	36.29±3.30^{d}	73.26±1.70^{d}
Positive group	391.86±4.10^{a}	60.14±2.12^{a}	106.00±2.71^{a}
Wheat germ (5%)	339.71±1.70^{b}	53.29±3.04^{b}	93.29±2.69^{b}
Wheat germ (10%)	329.29±2.81^{c}	45.86±2.48^{c}	82.00±2.65^{c}
Wheat germ (15%)	316.14±3.18^{d}	38.57±4.69^{d}	73.57±2.64^{d}

Means in each column with different superscript letters differ significantly at p<0.05. A uses harmonic mean sample size = 7 rats

Table 3: Serum concentrations of LDL-C, HDL-C and VLDL-C as well as atherogenic index in rats fed cholesterol-diet

	Parameters as Mean±SD			
Groups	LDL-C (mg/dL)	HDL-C (mg/dL)	VLDL-C (mg/dL)	AI
Negative group	37.89±3.14^{d}	28.14±1.77^{ab}	7.26±0.66^{d}	0.11±0.01^{d}
Positive group	72.43±3.91^{a}	21.57±2.15^{d}	12.03±0.42^{a}	0.46±0.02^{a}
Wheat germ (5%)	58.34±2.42^{b}	24.26±2.63^{c}	10.51±0.51^{b}	0.35±0.07^{b}
Wheat germ (10%)	43.57±4.27^{c}	26.26±1.80^{bc}	9.17±0.50^{c}	0.24±0.04^{c}
Wheat germ (15%)	36.86±2.57^{d}	29.00±1.63^{a}	7.71±0.94^{d}	0.12±0.04^{d}

Means in each column with different superscript letters differ significantly at p<0.05. A uses harmonic mean sample size = 7 rats

Table 4: Serum concentrations of GOT, GPT and ALP in rats fed cholesterol-diet

	Parameters as Mean±SD		
Groups	GOT (U/L)	GPT (U/L)	ALP (IU/L)
Negative group	13.14±1.07^{d}	10.29±1.11^{d}	35.15±2.91^{b}
Positive group	23.86±1.35^{a}	18.86±2.12^{a}	47.29±2.69^{a}
Wheat germ (5%)	20.00±1.41^{b}	14.71±2.06^{b}	35.43±2.07^{b}
Wheat germ (10%)	16.29±1.50^{c}	12.57±1.62^{c}	34.71±2.43^{b}
Wheat germ (15%)	14.29±0.95^{d}	11.00±1.15^{cd}	33.00±2.65^{b}

Means in each column with different superscript letters differ significantly at p<0.05. A uses harmonic mean sample size = 7 rats

Data also show that rats fed cholesterol-diet had significant decrease in serum level of HDL-C at p<0.05 (21.57±2.15 mg/dL) as compared to the rats fed cholesterol-free diet (28.14±1.77 mg/dL). Feeding cholesterol-diets with added 5, 10 and 15% wheat germ caused significant increase in serum level of HDL-C at p<0.05 (24.26±2.63, 26.26±1.80 and 29.00±1.63 mg/dL, respectively) as compared to the positive control group (21.57±2.15 mg/dL).

With regard to serum levels of VLDL-C, results revealed that positive control group had significant increase in serum levels of VLDL-C at p<0.05 (12.03±0.42 mg/dL) as compared to the negative control group (7.26±0.66 mg/dL). Groups feeding different levels of wheat germ had significant decrease in serum level of VLDL-C at p<0.05 (10.51±0.51, 9.17±0.50 and 7.71±0.94 mg/dL, respectively), compared to the positive control group (12.03±0.42 mg/dL).

Tabulated results demonstrated that mean±SD value for atherogenic index of positive control group was significantly increased at p<0.05 (0.46±0.02) as compared to the negative control group (0.11±0.01). However, rats fed cholesterol-diets formulated with different levels of wheat germ had significant decrease in atherogenic index at p<0.05 (0.35±0.07, 0.24±0.04 and 0.12±0.04, respectively) as compared to the positive control group (0.46±0.02).

Liver functions: Results in Table 4 revealed that positive control group had significant increases in serum levels of GOT, GPT and ALP at p<0.05 (23.86±1.35 U/L, 18.86±2.12 U/L and 47.29±2.69 IU/L, respectively) as compared to the normal control group (13.14±1.07 U/L, 10.29±1.11 U/L and 35.15±2.91 IU/L, respectively). Whereas, feeding groups on different levels of wheat germ (5, 10 and 15%) had significant decreases in serum levels of GOT, GPT and ALP at p<0.05 as compared to positive control group.

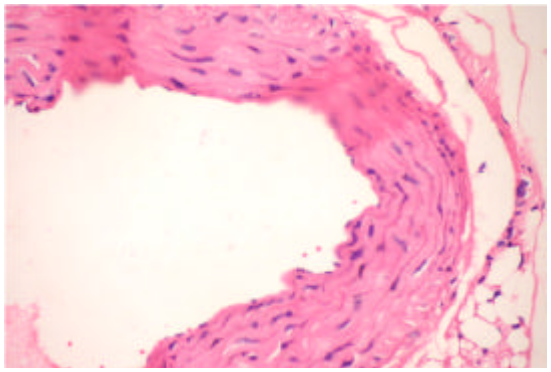

Fig. 1: Aorta of normal control rats showing no histopathological changes (H and E x 400)

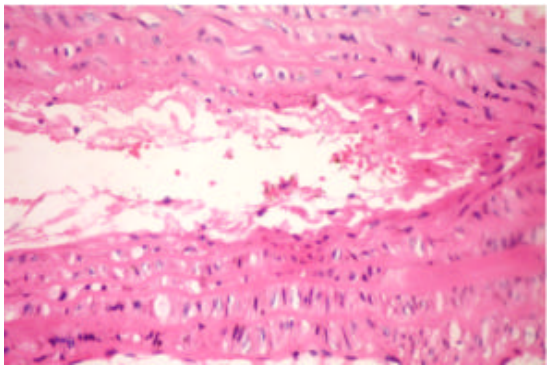

Fig. 2: Aorta of positive control rats showing vaculations of tunica media as well as narrowing in the lumen (H and E x 400)

Histopathological examination

Aorta: Histopathological examination of aorta from rats fed cholesterol-free diet revealed no histopathological changes as shown in Fig. 1. Aorta of rats fed cholesterol-containing diet had vaculations of tunica media and narrowing in the lumen as shown in Fig. 2,

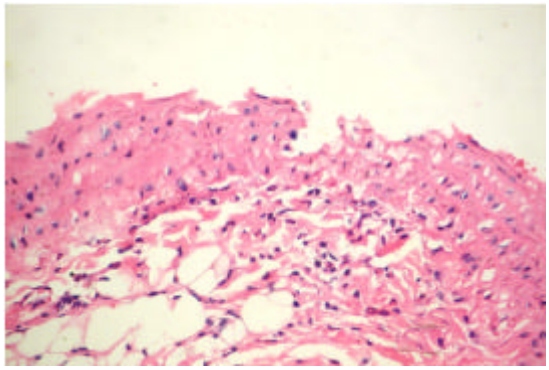

Fig. 3: Aorta of positive control rats showing focal necrosis of tunica intima and tunica media associated with few leucocytic cells infiltration (H and E x 400)

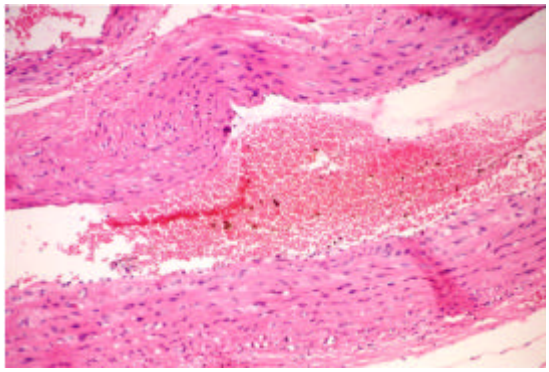

Fig. 4: Aorta of treated rats with 5% wheat germ showing slight thickening in the wall (H and E x 200)

as well as focal necrosis of tunica intima and tunica media associated with few leucocytic cells infiltration as shown in Fig. 3. Some sections of aorta from rats fed cholesterol-diet with added 5% wheat germ revealed no histopathological changes. Other sections of the same group showed slight thickening in the wall as shown in Fig. 4. Aorta sections of rats fed cholesterol-diet with added 10 and 15% wheat germ show normal histological structure.

Heart: Examination heart sections of normal rats show normal histological structure as shown in Fig. 5. In contrast, heart sections of positive rats revealed vaculations of cardiac myocytes associated with intramuscular edema as shown in Fig. 6. Examined heart sections of treated rats with 5% wheat germ show few leucocytic cells infiltration as shown in Fig. 7, however other sections revealed no histological structure. Heart of rats from treated group with 10 and 15% wheat germ show normal histological structure.

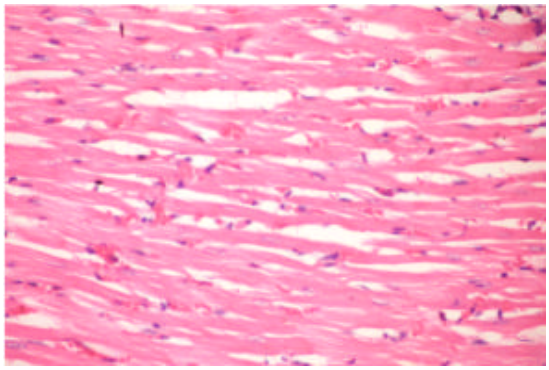

Fig. 5: Heart of normal control rats from negative group showing no histopathological changes (H and E x 400)

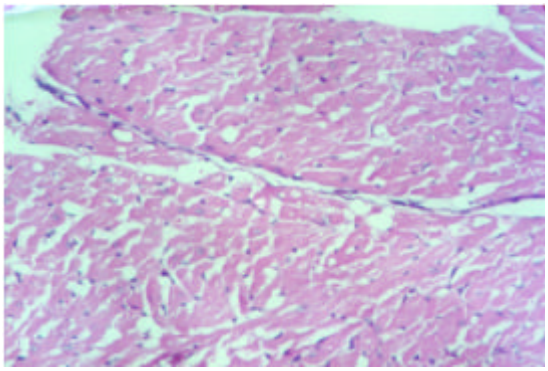

Fig. 6: Heart of rats from positive group revealed vaculations of cardiac myocytes associated with intramuscular edema (H and E x 400)

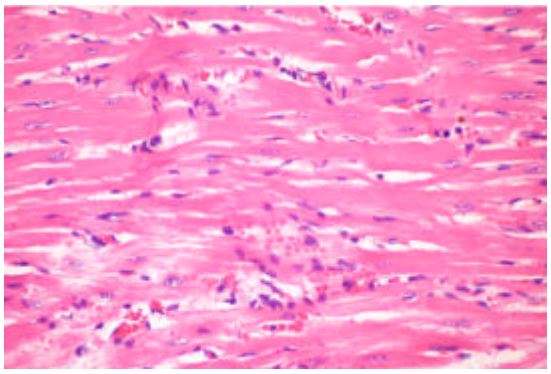

Fig. 7: Heart of rats from treated groups with 5% wheat germ showing few leucocytic cells infiltration (H and E x 400)

Liver: Histopathological examination of livers from normal rats revealed normal histological structure of hepatic lobule as shown in Fig. 8. Liver sections of positive rats show cytoplasmic vacuolization of

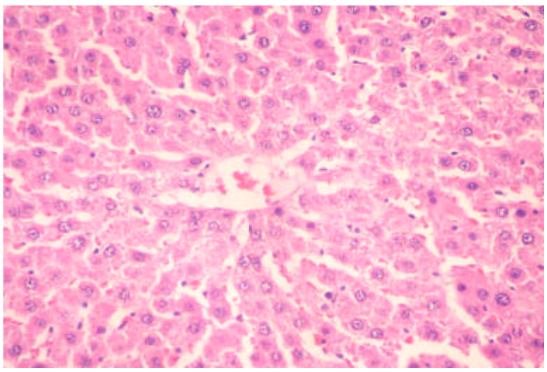

Fig. 8: Liver of negative control rats showing the normal histological structure of hepatic lobular (H and E x 200)

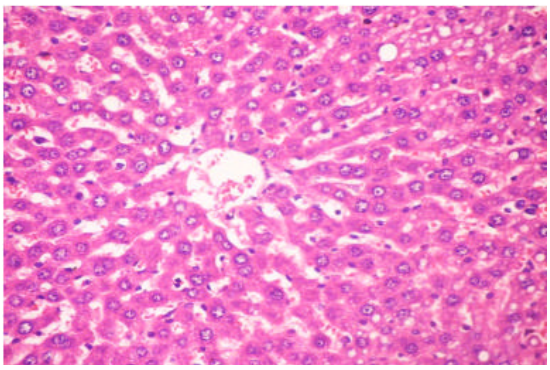

Fig. 9: Liver of positive control rats showing cytoplasmic vacuolization of hepatocytes (H and E x 400)

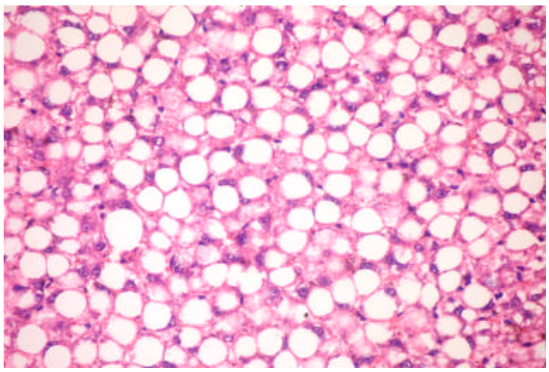

Fig. 10: Liver of positive control rats showing fatty change of hepatocytes (H and E x 400)

hepatocytes as shown in Fig. 9 and fatty changes of hepatocytes as shown in Fig. 10. Examined liver sections of treated rats with 5% wheat germ revealed fatty changes of hepatocytes as shown in Fig. 11. Small vacuoles in the cytoplasm of hepatocytes was observed in some examined sections from treated rats with 10%

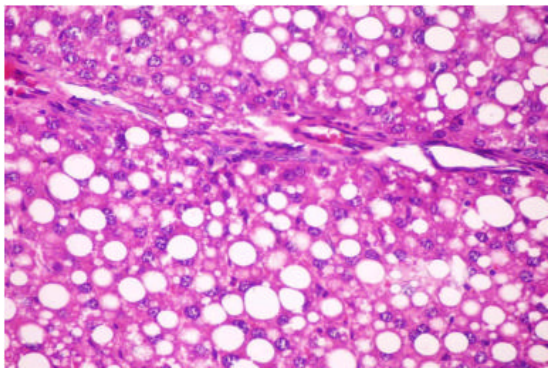

Fig. 11: Livers of treated rats with 5% wheat germ showing fatty change of hepatocytes (H and E x 400)

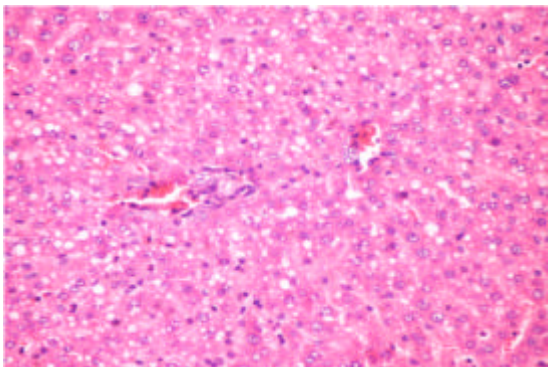

Fig. 12: Liver of treated rats with 10% wheat germ showing small vacuoles in the cytoplasm of hepatocytes (H and E x 400)

wheat germ as shown in Fig. 12, whereas, other sections showed no histological changes. Livers of rats treated with 15% wheat germ revealed apparent normal hepatocytes.

DISCUSSION

The present study aimed to investigate the effectiveness of different levels of wheat germ (5, 10 and 15%) on the protective of hypercholesteremic and atherosclerosis as risk factors of coronary heart disease in rats fed cholesterol-diet. The biomarkers used in this study provide the measures of atherosclerosis exposure in rats as an area of high risk for development of cardiovascular disease.

The present data revealed that change in food intake and feed efficiency ratio was not significantly decreased. This effect might be attributed to the fiber content of these diets, since possibly due to their similar cellulose contents (Gregorio *et al*., 2001).

However, the change in body weight gain might be attributed to lower food intake in positive group.

The present results revealed that rats fed cholesterol-diet (positive control group) had significantly increased in serum levels of Total Lipids (TL), Triglycerides (TG), Total Cholesterol (TC), LDL-C, VLDL-C, GOT, GPT and ALP and significantly decreased in serum level HDL-C as compared to that fed cholesterol free-diet (negative control group). The increases in serum levels of TL, TG, TC, LDL-C and VLDL-C as well as the reduction in levels of HDL-C could be increased the incidence of atherosclerosis which was represented by the increase in atherogenic index. These results were confirmed by histopathological examination of aorta, heart and liver, which showed that rats fed cholesterol-diet had vaculations of tunica media and narrowing in the lumen, as well as focal necrosis of tunica intima and tunica media associated with few leucocytic cells infiltration in aorta, vaculations of cardiac myocytes associated with intramuscular edema and cytoplasmic vacuolization of hepatocytes as well as fatty changes of hepatocytes. The increase in these parameters in positive rats might be attributed to that atherosclerosis is a heterogeneous combined change of the intima, the inner vascular layer. During this process, lipid accumulation and rebuilding of intercellular matrix occur with a predominance of fibrous tissue in the medial layer of the vascular wall. This is caused by metabolic abnormalities, alter structural properties of the vascular wall, hemodynamic factors and the changes in particular blood components (Horejsi, 2000).

The present results agreed with Witztum and Steinberg (1991) who reported that atherosclerosis is a multifactorial disease that represents the primary worldwide cause of death. Elevated plasma LDL cholesterol concentrations and low levels of HDL are associated with accelerated atherosclerosis. These results were confirmed with Friedman and Young (1997) who demonstrated that cholesterol assay are used to screening for atherosclerotic risk. HDL-C plays an important part in the removal of cholesterol from tissues and its transportation to the liver for removal as bile acids. Decreased serum HDL-C concentrations are positively correlated with the incidence of atherosclerosis disease. Moreover, Forester (2001) showed that high plasma levels of triglyceride and LDL-C being to be a risk factor for atherosclerosis and Coronary Heart Disease (CHD). Recent study revealed that a hypercholesterolemia combined with a marked hypertriglyceridemia leads to a moderate contractile dysfunction in heart of rats (Onody *et al*., 2003). Furthermore, Sheyla *et al*. (2005) showed that serum levels of cholesterol was increased and HDL-cholesterol reduced significantly in the experimental groups fed cholesterol rich-diet as compared to control. On the other hand, the elevation in liver enzymes may be attributed to their release from the cytoplasm into the blood circulation after rupture of the plasma membrane, cellular damage and infarcts in the myocardium. Tebib *et al*. (1994) reported that lipase is responsible on the transforms of VLDL in LDL-cholesterol and the activity of this enzyme increased in hypercholesterolemic animals. Furthermore, the uptake of LDL-cholesterol is dependent on receptors in plasmatic membrane (Berg *et al*., 2002). This may be happened in hepatic cells of the animal fed cholesterol-supplemented diets, justifying their higher LDL-cholesterol concentration. This effect could be related to approximately 80% of GOT in hepatocytes appear to be located in mitochondria, whereas GPT is thought to be predominantly no mitochondrial and it has been postulated that in mild hepatocellular injury, when the hepatocytes plasmatic but not the mitochondrial membrane is damaged, cytoplasmatic GOT and GPT are released into serum with more severe hepatocellular injury, mitochondrial membrane damage may result in the release of mitochondrial GOT and GPT (Pincus and Schaffner, 1996). These results were in accordance with Young (2001) who reported that GOT is a cellular enzyme found in high concentration in heart muscle and liver cells. GPT is a cellular enzyme found in high concentration in liver and kidney, it is used in the diagnosed of liver disease. GPT are used in conjunction with GOT to aid in the diagnosed of infarcts in the myocardium.

With regard to the effect of wheat germ, the present results revealed that rats fed cholesterol-diet with added 5 and 10% wheat germ had no significant change in food intake, while rats treated with 15% wheat germ had significant decreased in food intake as compared to the positive control rats. However, deferent levels of wheat germ caused significant increased body weight and feed efficiency ratio as compared to the rats fed cholesterol-diet. The decreased in food intake might be related to the fiber content of wheat germ. The increased in body weight gain and feed efficiency ratio may be related to the improvement in metabolic process as effect of wheat germ.

Rats fed cholesterol-diet and containing different levels of wheat germ had significantly decreased in serum levels of TL, TG, TC, LDL-C, VLDL-C, GOT, GPT and ALP, whereas, HDL-C was significantly increased as compared to the positive control group. The decrease in serum levels of the above mentioned parameters was more detectable with increased the level of wheat germ. The mean±SD values of atherogenic index were significantly decreased in the groups fed diet formulated diet with different levels of wheat germ.

Therefore, feeding different levels of wheat germ decrease the elevation in serum levels of TL, TG, TC, LDL-C, VLDL-C, GOT, GPT and ALP and decrease the incidence of atherosclerosis which was represented by the decreased in atherogenic index. Consequently, administration of wheat germ could prevent or decreased the incidence of hypercholesteremic and

atherosclerosis as risk factors of coronary heart disease. These results were confirmed by histopathological examination of aorta and heart, which revealed that rats fed cholesterol-diet formulated with 5% wheat germ revealed slight thickening in the wall of aorta and few leucocytic cells infiltration of some section of heart, whereas, other sections revealed no histological changes. Aorta and heart sections of rats fed cholesterol-diets formulated with 10 and 15% wheat germ show normal histological structure. The present results agreed with previous study established that the addition of 7% wheat germ to a high-fat and high-cholesterol diet improved lipoprotein in rats (Lairon *et al*., 1987). Soluble protein components of wheat germ are known to inhibit pancreatic lipase activity (Lairon *et al*., 1985).

In rats, the absorption of triacylglycerol and cholesterol was delayed and reduced by wheat germ and other wheat fractions in part as a result of the inhibition of pancreatic lipase and the reduction in triacylglycerol lipolysis (Borel *et al*., 1989). In addition to Louts *et al*. (1991) showed that wheat germ play a beneficial role in the dietary management of hyperlipidemia subjects. In the hypercholesteremic and hyperlipidemia subjects, total plasma cholesterol, VLDL-C and triglycerides were significantly decreased. Thus, the plasma HDL/total cholesterol ratio, apoprotein B and A1 were significantly decreased. Cara *et al*. (1991) who showed that serum triglyceride response was lower significantly in the presence of wheat germ in rats fed cholesterol-diet.

Moreover, Cara *et al*. (1992) reported that inclusion of wheat germ in a meal reduced plasma chylomicron cholesterol concentrations by 27.1% over several hours in subjects. These data suggest that wheat germ may lower circulating cholesterol or at least delay the absorption of cholesterol. Because wheat germ contains fiber, it has been thought that some effects on cholesterol metabolism might be mediated by dietary fiber.

On the other hand, the decrease in serum levels of the above mentioned parameter and decreased or preventive the incidence of atherosclerosis of wheat germ may be attributed to its content of antioxidant vitamin E. Previous study reported that the oxidative modification of LDL-C can be inhibited by antioxidants such as vitamin E (Naruszewicz *et al*., 1992; Viana *et al*., 1996).

In addition to, wheat germ has a high content of phytosterols relative to total fat, it contained 10.3% fat by wt and 4.1 mg phytosterols/g. Phytosterols intrinsic to wheat germ are biologically active and have a prominent role in reducing cholesterol absorption. Consequently, reducing cholesterol absorption would be lower serum cholesterol level. Phytosterols either in their free or esterified form, decrease blood levels of total cholesterol and LDL-cholesterol through reduction of cholesterol absorption (Richard *et al*., 2003).

In vitro studies results showed that plant sterols are effective in preventing hyper-proliferation of vascular smooth muscle cell that play a role in atherosclerosis development (Awad *et al*., 2001). Animal studies revealed that plant sterols also have antiatherogenicity activity and decreased plaque accumulation in coronary arteries within the ascending aorta (Ntanios *et al*., 1998). Another possible effect of plant sterols is their antioxidant activity as reported by (Wang *et al*., 2002) who suggested that the antioxidant activity may be in part related to sterol content.

Conclusion: The present study concluded that wheat germ could prevent or decreased the incidence of hypercholesteremic and atherosclerosis as risk factors of coronary heart disease, especially at higher levels.

REFERENCES

Assmann, G., H. Schulte and A. von Eckardstein, 1996. Hypertriglyceridemia and elevated lipoprotein (a) are risk factors for major coronary events in middle-aged men. Am. J. Cardiol., 77: 1179-1184.

Atwell, W.A., 2001. Wheat Flour. St. Paul, MN: Eagan Press.

Awad, A.B., A.J. Smith and C.S. Fink, 2001. Plant sterols regulate rat vascular smooth muscle cell growth and prostacyclin release in culture. Prostaglandins Leukot Essent Fatty Acids, 64: 323-330.

Berg, J.M., J. Tymoczko and L. Stryer, 2002. Biochemistry. 5th Edn., New York; Freeman and Company.

Borel, P., D. Lairon, M. Senft, M. Chautan and H. Lafont, 1989. Wheat bran and wheat germ: Effect on digestion and intestinal absorption of dietary lipids in the rat. Am. J. Clin. Nutr., 49: 1192-1202.

Cara, L., P. Borel, M. Armand, M. Senfit, M. Riottot, D. Lairon and J. Ferezou, 1991. Effects of increasing levels of raw or defatted wheat germ on liver. Feces and plasma lipids and lipoproteins in the rat. Nutr. Res. Elmsford, N.Y.: Pergamon Press, 11: 907-916.

Cara, L., C. Dubois and P. Borel, 1992. Effects of oat bran, rice bran, wheat fiber and wheat germ on postprandial lipidemia in healthy adults. Am. J. Clin. Nutr., 55: 81-88.

Carleton, H., 1979. Histological techniques, 4th Edn., London, Oxford University Press, New York, USA, Toronto.

Cohen, A.C., 2003. Insect Diets: Science and Technology. CRC Press.

Dobiasova, M. and J. Frohlich, 2001. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). Clin. Biochem., 34: 583-588.

Forester, J.S., 2001. Triglycerides: Risk factor or fellow traveler? Curr. Opin. Cardiol., 16: 261-264.

Friedman, A. and D. Young, 1997. Effects of diseases on clinical laboratory tests, 4th Edn., AACC Press.

Friedwald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Gregorio, S.R., M.A. Areas and F.G. Reyes, 2001. Dietary fibers and cardiovascular disease. 22: 109-120.

Holland, B., A.A. Welch, I.D. Unwin, D.H. Buss, A.A. Paul and D.A. Southgate, 1991. The composition of foods. The Royal Society of Chemistry Cambridge. In McCance and Widdowsons.

Horejsi, R.C., 2000. Apolipoproteins and atherosclerosis. Apolipoprotein E and apolipoprotein (a) as candidate genes of premature development of atherosclerosis. Physiol. Res., 49 (suppl.1): S63-S69.

Lairon, D., P. Borel, E. Termine, R. Grataroli, C. Chabert and J.C. Hauton, 1985. Evidence for a proteinic inhibitor of pancreatic lipase in cereals, wheat bran and wheat germ. Nutr. Rep. Int., 32: 1107-1113.

Lairon, D., C. Lacombe and P. Borel, 1987. Beneficial effect of wheat germ on circulating lipoproteins and tissue lipids in rats fed a high cholesterol-containing diet. J. Nutr., 117: 838-845.

Louts, C., B. Patrick, A. Martine, E. MicheLlesent, L. Huguette, P. Henri, P. Anne-Marie, B. Daniele, L. Christianne and L. Denis, 1991. Plasma lipid lowering effects of wheat germ in hypercholesterolemic subjects. Plant Foods Human Nutr., 41: 135-150.

Naruszewicz, M., E. Selinger and J. Davignon, 1992. Oxidative modification of lipoprotein (a) and the effect of beta carotene. Metabolism, 41: 1215-1224.

Ntanios, F.Y., P.J. Jones and J.J. Frohlich, 1998. Dietary sitostanol reduces plaque formation but not lecithin cholesterol acyl transferase activity in rabbits. Atherosclerosis, 138: 101-110.

Nichelatti, M. and M. Hidvegi, 2002. Experimental and clinical results with Avemar (a dried extract from fermented wheat germ) in animal cancer models and in cancer patients. Nogyogyaszati Onkologia; 7: 180-185.

Onody, A., C. Csonka, Z. Giricz and P. Ferdinandy, 2003. Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. Cardiovasc. Res., 58: 663-670.

Pincus, M.R. and J.R. Schaffner, 1996. Assessment of liver function in clinical diagnosis and management by laboratory methods. Philadelphia; W.B. Saunders Company.

Reeves, P.G., F.H. Nielson and G.C. Fahmy, 1993. Reports of the American Institute of Nutrition, adhoc-wiling committee on reformulation of the AIN 93. Rodent diet. J. Nutr., 123: 1939-1951.

Richard, E., J. Ostlund, B.R. Susan and F.S. William, 2003. Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. Am. J. Clin. Nutr., 77: 1385-1389.

Sheyla, L.M., H. de Paula, L.P. Maria, C.S. Rinaldo, L. Eduardo, A.C. Deoclecio and E.S. Marcelo, 2005. Dietary models for inducing hypercholesterolemia in rats. Brazilian Arch. Biol. Technol., 48: 203-209.

Szapary, P.O. and D.J. Rader, 2004. The triglyceride-high-density lipoprotein axis: An important target of therapy? Am. Heart. J., 148: 211-221.

Tebib, K., J.M. Rouanet and P. Besancon, 1994. Effect of grape seed tannins on the activity of some rat intestinal enzyme activities. Enzyme Protein, 48: 51-60.

Viana, M., C. Barbas, B. Bonet, M. Castro, M.V. Fraile and E. Herrera, 1996. *In vitro* effects of flavonoid-rich extract on LDL oxidation. Atherosclerosis, 123: 83-91.

Witztum, J.L. and D. Steinberg, 1991. Role of oxidized low density lipoprotein in atherogenesis. J. Clin. Invest., 88: 1785- 1792.

Wang, T., K.B. Hicks and R. Moreau, 2002. Antioxidant activity of phytosterols, oryzanol and other phytosterol conjugates. J. Am. Oil Chem. Soc., 79: 1201-1206.

Yarnell, J.W., C.C. Patterson, P.M. Sweetnam, H.F. Thomas, D. Bainton and P.C. Elwood, 2001. Do total and high density lipoprotein cholesterol and triglycerides act independently in the prediction of ischemic heart disease? Ten-year follow-up of Caerphilly and Speedwell Cohorts. Arterioscler Thromb Vasc Biol., 21: 1340-1345.

Yiqiang, G., S. Aidong, N. Yuanying and C. Tongyi, 2001. Study and development of a defatted wheat germ nutritive noodle. Eur. Food Res. Technol., 212: 344-348.

Young, D.S., 2001. Effects of disease on clinical Lab. tests, 4th Edn., AACC press.