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Assessment of varying concentrations of wheat germ oil in defatted cookies on serum lipid and protein profile of albino rats.

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ABSTRACT

The cookies prepared from different flour blends were assayed through albino rats for nutritional quality to explore the protein bioavailability. The biochemical evaluation of albino rats indicated that serum concentrations of total cholesterol, LDL and thiobarbituric acid reactive substances (TBARS) value were affected significantly due to the variation of diets. However, serum HDL and triglycerides concentrations showed non-significant differences due to diets. The lowest total serum cholesterol was observed in the rat group fed on cookies prepared from 100% WGO while

the highest cholesterol (107.58 mg/dl) was possessed by the group fed on control diet (100% normal shortening). Similarly, the highest value for LDL was observed in the rat group fed on diet containing 100% WGO while the lowest by the group fed on control diet. The serum TBARS value of rats decreased as the level of WGO increased in the diets. The rat group fed with 100% WGO based cookies possessed the lowest serum TBARS value as compared to all other treatment. The decrease in TBARS value showed that resistance of serum to oxidation reduced by the incorporation of WGO in the rat diets. The results portrayed that incorporation of DFWG and WGO in the cookies improved biochemical as well as nutritional parameters of cookies.

Key words: Defatted wheat germ oil (DFWG), cookies, lipid profile, protein quality

INTRODUCTION

Wheat (*Triticum aestivum*) is a major cereal crop grown in many parts of the world. On global basis, wheat and rice accounts for over 50% of the total cereal production in world (Lookhart and Bean, 2000). The wheat grain consists of three distinct parts, the bran, the germ and the endosperm. The distribution of these parts in wheat kernel is as: 82 to 83% endosperm, 12 to 14% bran and 2 to 4% germ (Posner, 2000). The wheat germ is the embryo of wheat, which has reproductive function and separated during milling from endosperm because it adversely influences the keeping quality as well as the processing quality of the wheat flour. The wheat germ presently is mainly used in animal feed formulations. So, the wheat germ, a rich source of nutrients is not amply, rationally, and efficiently being utilized (Ge *et al*. 1999). Most of the nutrients in the wheat grain with the exception of starch are concentrated in the germ. It is an excellent source of vitamins, minerals, dietary fiber, calories, proteins, and some functional micronutrients (Shurpalekar and Rao, 1977). The wheat germ not only possesses proteins of high biological value but also its oil is of desirable fatty acid profile and richest natural source of tocopherols (Barns and Tayler, 2006). Due to its composition, the wheat germ is praised as "the natural nutrient treasure-house and life source of mankind" because of its high nutritive value and palatability. The wheat germ contains about 11-14% oil (Singh and Rice, 1980), which can be extracted from wheat germ either by solvent extraction (90% recovery) or by mechanical pressing (50% recovery) (Gomez and Ossa, 2000). The oil in wheat germ has been found to reduce plasma and liver cholesterol in animals (Kahlon, 1989) due to presence of highly beneficial polyunsaturated fatty acids and bioactive compounds like octacosanol and tocopherols (Saito and Yamauchi, 1990). The wheat germ oil comprises of high content of polyunsaturated fatty acids among which about 80%, mostly linoleic (18:2) and linolenic (18:3) fatty acids (Wang and Johnson, 2001). These fatty acids are essential and also are precursors of a group of hormones called prostaglandins, which play an important role in muscle contractions and in the proper healing of inflammatory processes (Cultate, 1995). Grundy and Denke (1990) estimated that increasing linoleic acid intake lowers cholesterol about half as much as saturated fatty acids increase it. The wheat germ oil also possesses high content of octacosanol a 28 carbon long-chain saturated primary alcohol found in a number of different vegetable waxes, which improves exercise and physical performance (Hass, 2006). It has also been reported to reduce plasma cholesterol in humans (Xu *et al.,* 2007). The effects of octacosanol on hypercholesterolemia have been related to its LDL cholesterol decreasing and HDL cholesterol increasing trends (Varady

et al., 2003). The wheat germ oil contains the highest content of natural antioxidants i.e. tocopherols up to about 1850 mg/kg oil (Davis *et al.,* 1980). Wheat germ oil is more stable to oxidation or rancidity than many other oils due to the antioxidant properties of tocopherols (Haas, 2006). The mechanism of tocopherols for its antioxidant activity includes the transfer of a hydrogen atom from the 6–hydroxyl group on the chroman ring, and scavenging of singlet oxygen and other reactive species (Niki, 1989). The tocopherols can protect polyunsaturated fatty acids within the membrane and LDL, and inhibit smooth muscle cell proliferation and thus help in the reduction of heart disease, delay of Alzheimer's disease, and prevention of cancer (Meydani, 2000). The tocopherols contain four isomers i.e. α, β, γ and δ tocopherols but isomers α and γ are responsible for the antioxidant properties and cardiovascular health (Ruiz *et al.* 2002). Natural α-tocopherol is a potent lipid-soluble antioxidant carried in LDL and increases its resistance to oxidation and inhibits the proliferation of smooth-muscle cells in vitro and when added to plasma. Natural tocopherol is superior to the synthetic one not only for its biological function but also with respect to its dietary safety and public acceptance. The number of hypercholesterolemic people in Pakistan is increasing tremendously. The consumption of foods rich in natural antioxidants, which are potentially able to quench or neutralize excess radicals, may help to overcome these adverse causes. The importance of polyunsaturated fatty acids and natural antioxidants (tocopherols) in human diets and their substantial availability in wheat germ oil led to trial its suitability for the production of cookies and further investigate the role of its bioactive compounds in the lipid profile of albino rats. On the other hand, after extraction of oil from wheat germ, the defatted wheat germ is also highly nutritive value protein material, which contains about 30% protein (Ge *et al.,* 2000). The cereals possess some essential amino acids in relatively low concentrations. The first limiting amino acid in wheat is lysine, which demands that wheat should be supplemented with high lysine-rich proteins to provide a balanced diet (Pichardo *et al.,* 2003). The endosperm contains higher gluten content which is low in lysine however; albumins and globulins are rich in lysine and are located mainly in the bran and the germ parts of wheat kernel (Lookhart and Bean, 2000).

The population is suffering with malnutrition mainly due to protein deficiency. The prevalence of varying degree of protein deficiency in some vulnerable groups of population, due to intake of low quality and quantity of protein has been reported in Pakistan (Anon, 2004). The cereals contribute more than 60% to the total protein intake and provide calories to the people of Pakistan. The consumption of animal proteins in Pakistan is low because of its limited supply and high prices. Thus the incorporation of defatted wheat germ protein in bakery products can provide the required essential amino acids to cope this deficiency.

In Pakistan, little or no efforts have been made to eliminate or reduce the risk of diseases through diet therapy. There is a need to introduce such raw materials rich in nutrients in daily diet chart which can reduce the threat of protein deficiency, cancer and cardiovascular diseases. The wheat germ utilization in food products has not yet been explored. Keeping in view the nutritional importance and availability of wheat germ, the research study has been undertaken to achieve the following objectives:

- To establish the effect of wheat germ oil based cookies on serum lipid profile through efficacy studies in rats.

MATERIALS AND METHODS

The research work was conducted to study the effect of wheat germ oil based cookies on serum lipid profile through efficacy studies in rats. The wheat germ was the courtesy of Sunny Flour Mills, Lahore and straight grade flour was purchased from local market. The wheat germ and flour samples were packed in polypropylene bags and stored at room temperature for further analysis. The oil was extracted from wheat germ through solvent extraction technique by using n-hexane. The extracted oil was heated at 40°C to remove the last traces of solvent. The crude oil recovered in this way was kept in desicator over anhydrous calcium chloride for twenty four hours, so that the traces of moisture (if present) could be removed. The percentage recovery of oil was calculated by the formula:

$$\text{Wheat germ oil (\%)} = \frac{\text{Wt. of oil (g)}}{\text{Wt. of wheat germ sample (g)}} \times 100$$

The wheat germ, defatted wheat germ (DFWG) and wheat flour were analyzed for their proximate composition, minerals and amino acid content. The defatted wheat germ (DFWG) residue after oil extraction from collected germ was smashed and passed through a 200-mesh sieve to obtain DFWG flour, which was packed in polypropylene bags for further studies.

FLOUR BLENDS FORMULATION

The flour blends were prepared by incorporating DFWG flour in to wheat flour at different concentration levels for the preparation of high protein cookies. The wheat flour was replaced with DFWG flour for blends formulation at different levels as given here in Table 1.

Table 1. Formulation of DFWG supplemented flour blends

Treatments	Wheat Flour (%)	DFWG Flour (%)
T_0	100	0
T_1	95	5
T_2	90	10
T_3	85	15
T_4	80	20
T_5	75	25

The choice of the above levels of DFWG was based on the report of Dreuiter (1978) who reported the maximum level (25%) of wheat flour substitution for an acceptable baked product.

PREPARATION OF DFWG SUPPLEMENTED COOKIES

The cookies were prepared from DFWG blended flours according to the procedure given in AACC (2000) with some modifications. The basic ingredients used were 380 g of flour blend, 100 g

vegetable shortening, 225 g of granulated cane sugar, 21 g of beaten whole egg, 3.75 g of salt, and 1.8 g of baking powder. The dry ingredients were weighed and mixed thoroughly in a mixing bowl for 3 to 5 minutes. The shortening was added and rubbed-in until uniform mass is formed. The egg was added and dough thoroughly kneaded in a mixer for 5 minutes. The dough was rolled thinly on a sheeting board to a uniform thickness (8 mm) and cut out using a round scorn cutter to a diameter of 35 mm. The cut out dough pieces were baked on greased pans at 160°C for 15 minutes in baking oven. The cookies were cooled at room temperature (30°C) and packed in high density polyethylene bags and stored for two months.

BIOLOGICAL STUDY

PREPARATION OF DIETS

The cookies prepared from five treatments were dried and milled into fine powder using porcelain pestle and mortar. The diets were prepared by adding other ingredients in the cookie powder according to the ratio mentioned in Table 2. The diet prepared from the cookies containing 0% WGO (T_0) was taken as control.

HOUSING OF RATS

Nearly 50 young male Sprague Dawley rats weighing 60 to 80g were purchased from National Institute of Health (NIH), Islamabad, Pakistan. The rats were housed in five individual stainless steel screen cages and allowed free access to food and water. The cages were maintained in a room where temperature of 25°C and 50% relative humidity were maintained. The rats were fed on the freshly prepared diets (Table 2) and watered individually in separate cages on daily basis up to two months.

ANALYSIS OF RAT SERUM

The blood samples of the rats were collected at the end of experiment after two months. The rats were fasted overnight, anesthetized with diethyl ether and sacrificed and the blood samples were collected. The serum was separated by centrifugation at 3000 rpm for 15 minutes after allowing the blood to stand for at least 30 minutes at room temperature as explained by Uchida *et al*. (2001). Following biochemical analysis were carried out from the collected rat serum samples according to their respective methods given below.

TOTAL CHOLESTEROL

The cholesterol in the collected serum of individual rats of all groups was measured by liquid cholesterol CHOD–PAP as method described by Stockbridge *et al.* (1989) to find out the effect of individual diet on the cholesterol level of respective groups.

Diets	Ingredients							
	Cookies (g)	Corn starch (g)	Corn oil (g)	Glucose (g)	Min. mix (g)	Vit. mix (g)	Total (g)	Protein (%)
T_0	84.67	0.33	-	5.0	5.0	5.0	100	10
T_1	84.67	0.33	-	5.0	5.0	5.0	100	10

T_2	84.67	0.33	-	5.0	5.0	5.0	100	10
T_3	84.67	0.33	-	5.0	5.0	5.0	100	10
T_4	84.67	0.33	-	5.0	5.0	5.0	100	10

Table 2. Composition of experimental diets (WGO based cookies)

T0 = Diet prepared from cookies containing 0% WGO

T1 = Diet prepared from cookies containing 25% WGO

T2 = Diet prepared from cookies containing 50% WGO

T3 = Diet prepared from cookies containing 75% WGO

T4 = Diet prepared from cookies containing 100% WG

HIGH DENSITY LIPOPROTEIN (HDL)

The serum high density lipoprotein (HDL) was measured by HDL cholesterol precipitant method as described by Assmann (1979) to find out the impact of prepared diets on the HDL level of specified groups of rats.

TRIGLYCERIDES

The triglycerides in the collected serum of individual rats were measured by liquid triglycerides GPO - PAP method as described by Annoni *et al.* (1982).

STATISTICAL ANALYSIS

The data were interpreted by analysis of variance (ANOVA) using M-Stat C software package as described by Steel *et al.* (1997). ANOVA analysis tested the significance of the differences between samples at 5% level of significance. The Duncan's Multiple Range (LSD) test was used to determine the level of significance that existed between the mean values.

RESULTS AND DISCUSSION

TOTAL CHOLESTEROL

The statistical results for total serum cholesterol of rats fed with diet cookies supplemented with various levels of WGO are presented in Table 3. The results indicated that total cholesterol was significantly affected by the diets. The highest concentration of total cholesterol (107.58 mg/dl) was observed in the control group which was fed with cookies containing 100% normal shortening (Figure 1). The rats group fed on cookies of T_4 (100% WGO) possessed the lowest content of total cholesterol in serum. The results further showed that cookies of treatments T_2 (25% WGO) and T_3 (50% WGO) were statistically at par with respect to total cholesterol. Similarly, non-significant differences were observed between diets containing cookies of T_3 and T_4 for total cholesterol content of serum (Figure 1). It is evident from Figure 1 that total cholesterol decreased linearly with increasing level of WGO in the diets. The results in the present study demonstrated a reduction in total cholesterol by increasing WGO concentration in the test diets which have been experienced by Kahlon (1989). Previously *Car et al.* (1991) have also observed the reduction in total cholesterol of rats fed with raw wheat germ in their diets and attributed the reduction in total cholesterol to tocopherols, octacosanol and polyunsaturated fatty acids of wheat germ.

SOV	df	Total cholesterol	LDL	HDL	Triglycerides	TBARS value
Treatments	4	35.319**	25.655**	0.089NS	5.784NS	0.095**
Error	20	1.405	1.953	2.378	1.448	0.0021

Table 3. Mean squares for lipid parameters of rats fed on diets prepared from WGO based cookies

**Significant (P < 0.01)
LDL = Low density lipoprotein
HDL = High density lipoprotein
TBARS = Thiobarbituric acid reactive substances

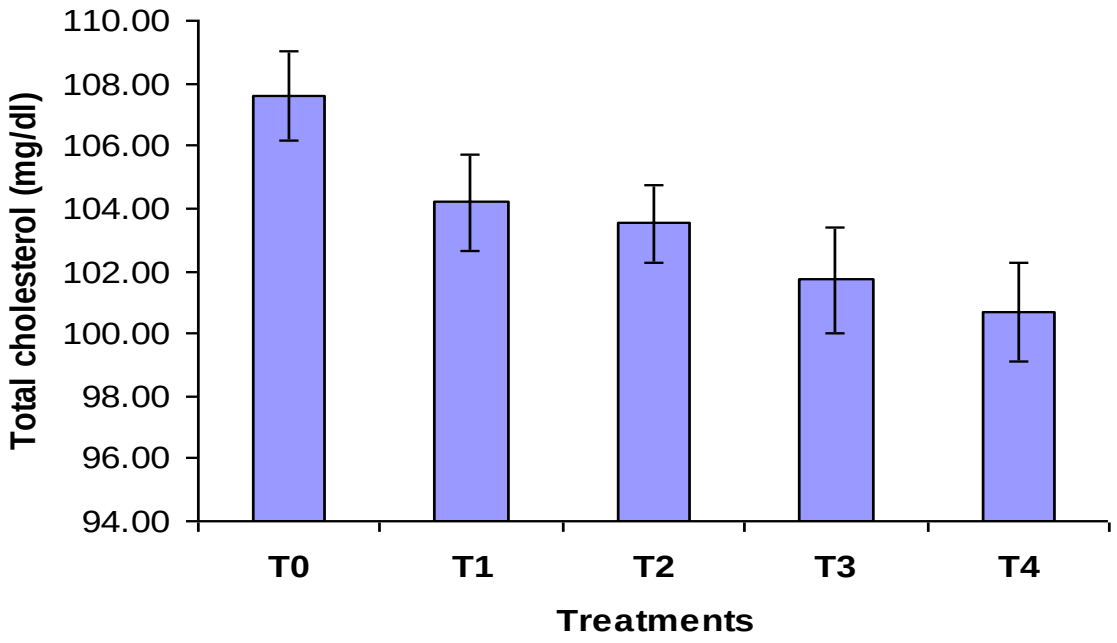

Figure 1. Effect of diets prepared from WGO based cookies on the total serum cholesterol of rats

T_0 = Rats group fed on cookies prepared with 0% WGO replacement (control)
T_1 = Rats group fed on cookies prepared with 25% WGO replacement
T_2 = Rats group fed on cookies prepared with 50% WGO replacement
T_3 = Rats group fed on cookies prepared with 75% WGO replacement

The decreasing trend of total cholesterol by test diets may thus be attributed to the polyunsaturated fatty acid (PUFA) and octacosanol content of WGO. Grundy and Denke (1990) estimated that increasing linoleic acid intake lowers cholesterol about half as much as saturated

fatty acids increase it. It is well known that normal shortening has a high concentration of saturated fats, particularly palmitic, myristic and lauric acids (Lai *et al.,* 1995). The results for higher total serum cholesterol content in the rats fed on control cookies diet (T_0) observed in this study are also in agreement with several investigators (McNamara, 1993) who attributed the rise in total cholesterol concentration in plasma of humans and experimental animals to the presence of high levels of myristic, palmitic and lauric acids in diets. The results of the present study are further supported by Xu *et al.* (2007) who observed same declining trend in the total cholesterol content of rat serum when they were fed with octacosanol doses with high fat diet. The results showed that supplementation of the high-fat diet with octacosanol led to a significant decrease in the total cholesterol level of rats. A significant decrease in the total cholesterol by the intake of cookies containing higher content of WGO may be thus attributed to their higher levels of poly unsaturated fatty acids and octacosanol content.

LOW DENSITY LIPOPROTEIN (LDL)

The analysis of variance regarding serum LDL content of rats fed with diets of cookies supplemented with various levels of WGO (Table 3) showed significant effect of diets on this parameter. It is evident from Figure 2 that the LDL content was found maximum in rats fed on diets of control cookies (100% normal shortening) while the lowest LDL content was observed in the group fed on cookies of T_4 (100% WGO). It is obvious from the results (Figure 2) that negative correlation existed between WGO concentration in the diet and LDL concentration in the serum of rats. The rats group fed with diets of cookies containing WGO in their formulation exhibited lower LDL values as compared to rats group fed with cookies of control diet (normal shortening).

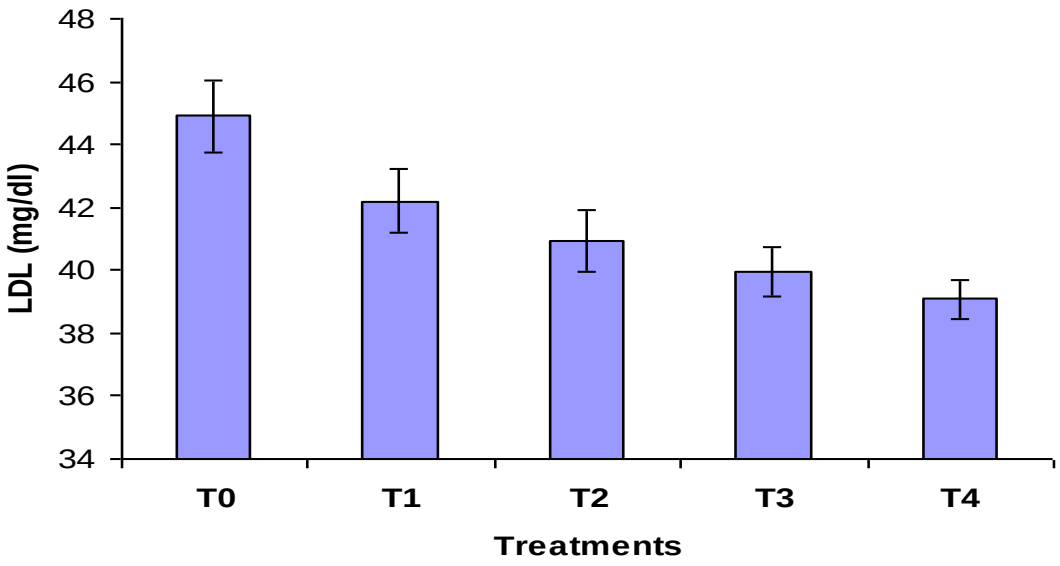

Figure 2. Effect of diets prepared from WGO based cookies on the serum LDL concentration of rats
T_0 = Rats group fed on cookies prepared with 0% WGO replacement (control)
T_1 = Rats group fed on cookies prepared with 25% WGO replacement
T_2 = Rats group fed on cookies prepared with 50% WGO replacement
T_3 = Rats group fed on cookies prepared with 75% WGO replacement
T_4 = Rats group fed on cookies prepared with 100% WGO replacement

The results of the present study demonstrated a reduction of about 15% in the LDL of rats group fed with diets composed of 100% WGO (T_4) which is in close agreement with the previous studies. The decrease in LDL by the supplementation of WGO in the rat's diets can be attributed to octacosanol, and polyunsaturated fatty acids (PUFA) content of WGO. Eisenmenger and Dunford (2008) have postulated that the LDL lowering effects of WGO could be attributed to the bioactive compounds present with special reference to octacosanol. Several clinical studies have shown that octacosanol may reduce LDL levels by 8% to 21% in patients with hypercholesterolemia (Pons *et al.,* 1992). Although the mechanisms of LDL-lowering effects of octacosanol are not fully understood, it has been suggested that octacosanol may increase lipid catabolism (Shimura *et al.,* 1987). It is possible that octacosanol increases the production and activities of lipoprotein and hepatic lipases and thereby reduces the levels of circulating LDL. Hence the reduction LDL content in the present study is well supported by the findings of different above mentioned workers.

HIGH DENSITY LIPOPROTEIN (HDL)

The statistical results indicated that serum HDL concentration of rats fed with various diets was not affected significantly by the diets (Table 3). The results in Figure 3 illustrated the effect of diets on HDL concentration of different groups of rats. It is obvious that all the groups of rats showed similar level of serum HDL. The rats groups fed on cookie diets containing WGO in their formulation however, showed a non significant differences in the serum HDL concentration as compared to the control diet (100% normal shortening).

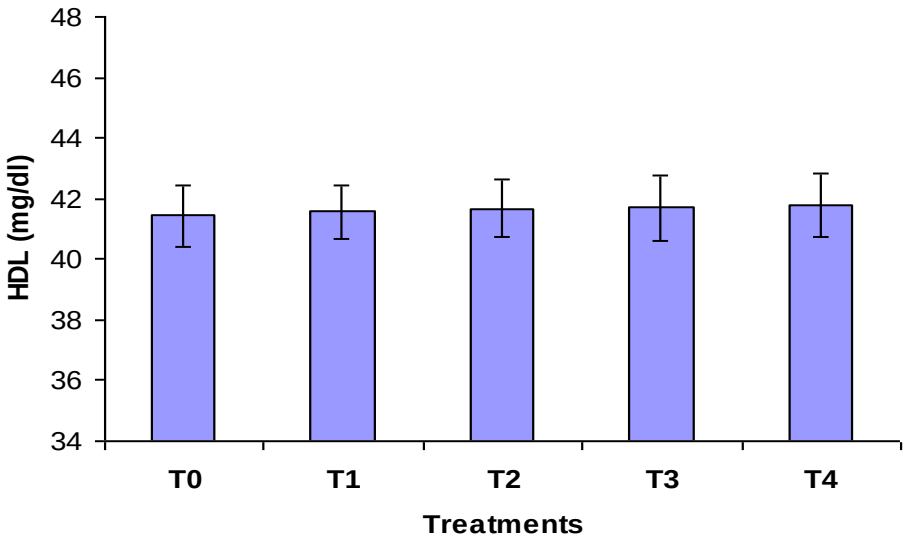

Figure 3. Effect of diets prepared from WGO based cookies on the serum HDL concentration of rats
T0 = Rats group fed on cookies prepared with 0% WGO replacement (control)
T1 = Rats group fed on cookies prepared with 25% WGO replacement
T2 = Rats group fed on cookies prepared with 50% WGO replacement
T3 = Rats group fed on cookies prepared with 75% WGO replacement
T4 = Rats group fed on cookies prepared with 100% WGO replacement

The results of the present study are corroborated with the previous findings of Cara et al. (1991) who observed similar non-significant change in serum HDL level by feeding the rats with raw wheat germ. The researchers stated that the composition of HDL might change more slowly than that of other lipoprotein particles like LDL and VLDL. Similarly, Chang and Huang (1998) studied the effect of different levels of dietary monounsaturated and polyunsaturated fatty acids on plasma and liver lipid concentrations and observed that the plasma very low density lipoprotein (VLDL) and low density lipoprotein (LDL) increased significantly in the high mono unsaturated fatty acid diet group, but high density lipoprotein (HDL) did not change significantly.

TRIGLYCERIDES

The analysis of variance regarding serum triglycerides showed a non-significant effect of diets (Table 3). The results in Figure 4 showed the similar response of all the diets towards level of triglycerides of different rat groups. However, it is evident from Figure 4 that higher serum triglyceride content was observed in the rat group fed with cookies of control diet (100% normal shortening), while the lower triglyceride content was recorded the groups fed with diets of cookies from T2 (50% WGO) and T3 (75% WGO). The results found in the present study are in close agreement with Cara et al. (1991), who observed the similar non-significant response for triglycerides concentrations from the rats when fed with different levels of raw wheat germ. They postulated that 20 g/day raw wheat germ was adequate for lowering plasma cholesterol but not for lowering plasma triglycerides and it was noteworthy that the magnitude of triglyceride reduction was not also as high as could be expected (Borel et al., 1989) after a 30 g/day raw wheat germ intake.

THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) VALUE

The susceptibility of lipoproteins to peroxidation was monitored by serum TBARS value of rats at the expiry of experiment. The statistical results regarding serum TBARS value of rats fed with diets of cookies supplemented with various levels of WGO (Table.3) demonstrated that the diets showed significant effect on the serum TBARS value. The highest TBARS value was recorded in rats fed on cookies of T0 (100% normal shortening) while the rats fed on cookies of T4 (100% WGO) possessed the lowest TBARS value at the end of the study (Figure 4).

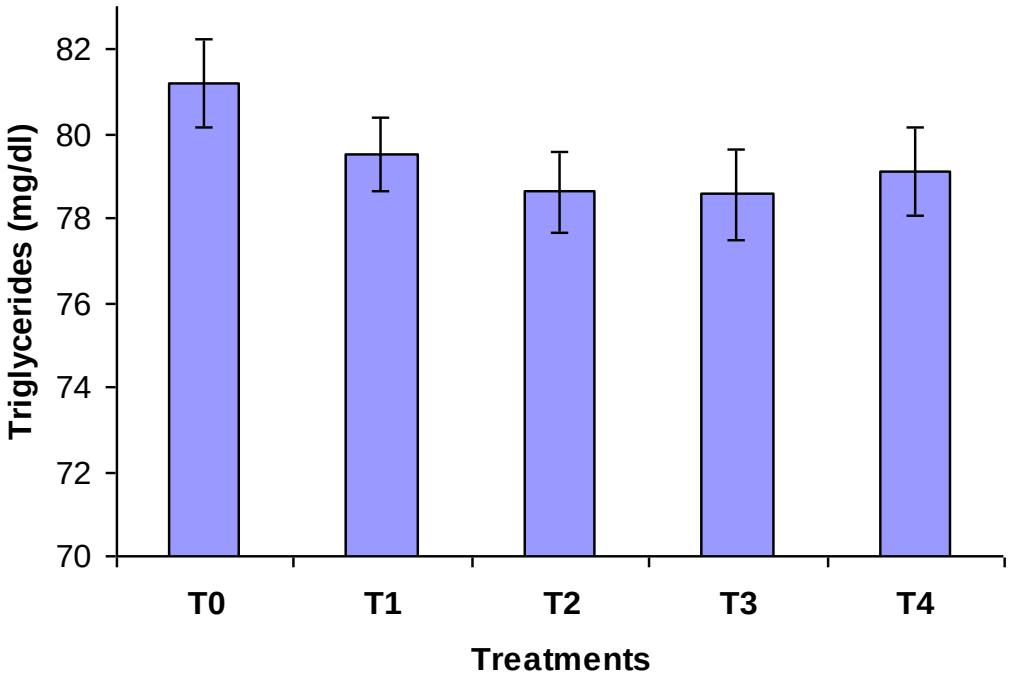

Figure 4. Effect of diets prepared from WGO based cookies on the serum triglycerides of rats

T_0 = Rats group fed on cookies prepared with 0% WGO replacement (control)

T_1 = Rats group fed on cookies prepared with 25% WGO replacement

T_2 = Rats group fed on cookies prepared with 50% WGO replacement

T_3 = Rats group fed on cookies prepared with 75% WGO replacement

T_4 = Rats group fed on cookies prepared with 100% WGO replacement

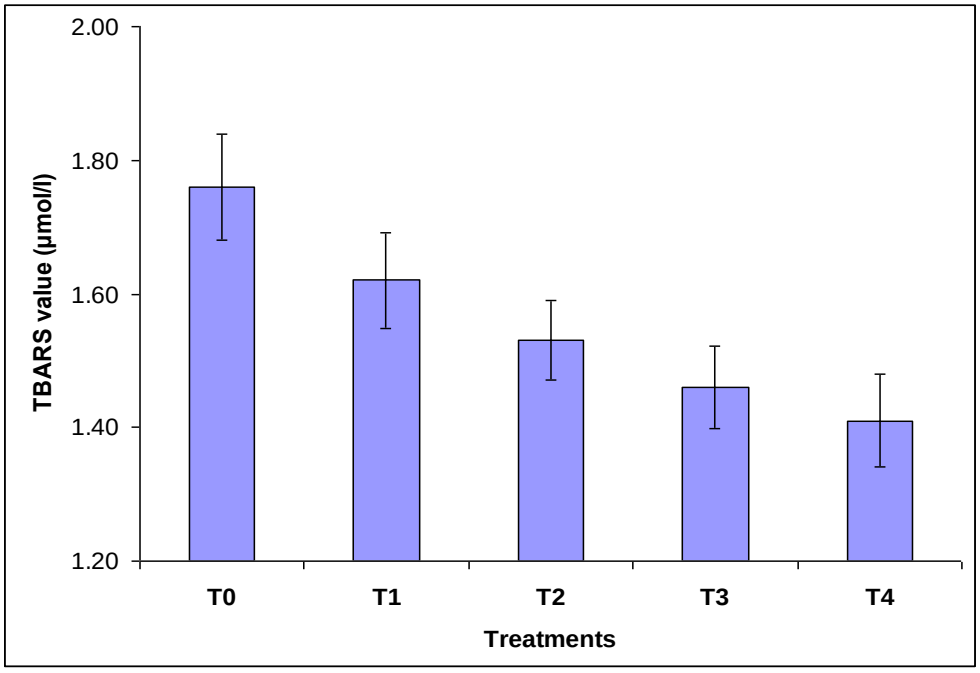

Figure 5. Effect of diets prepared from WGO based cookies on the TBARS value of rats

T0 = Rats group fed on cookies prepared with 0% WGO replacement (control)
T1 = Rats group fed on cookies prepared with 25% WGO replacement
T2 = Rats group fed on cookies prepared with 50% WGO replacement
T3 = Rats group fed on cookies prepared with 75% WGO replacement
T4 = Rats group fed on cookies prepared with 100% WGO replacement

The results revealed that serum TBARS value was decreased with the increasing level of WGO in the diets rats groups. The decreasing trend in TBARS value was consistent upto cookies of T2 (50% WGO) however, non-significant differences were observed between cookies of T3 (75% WGO) and T4 (100% WGO), which showed the same effect of both treatments on the serum TBARS value of rats. In the present study, serum TBARS value may be used as a marker of lipid peroxidation and formation of free radicals in the rats. There is increasing evidence that the initiation of atherosclerosis is related to free radical reactions, lipid peroxidation, and oxidative modification of low-density lipoproteins (Stam et al., 1989). Current findings showed that TBARS levels (Figure 5) reduced in rats groups fed on cookies containing WGO as compared to the control group. The resistance to lipid peroxidation in the rats fed with WGO based diets may be due to the higher concentrations of tocopherols present in WGO which is in agreement with the previous findings of Alessandri et al. (2006) who stated that in patients with hypercholesterolemia, wheat germ oil supplementation was associated with parallel reduction of oxidative stress depicted by TBARS value. It is well documented that tocopherols possessed the ability to suppress lipid peroxidation due to its antioxidant activity. Morel and Chisolm (1989) found that tocopherol treatment of diabetic rats inhibited the oxidation of serum lipids and reduced the TBA reactivity in serum. Other researchers have also identified fatty acid composition, ubiquinol-10 and hydroperoxide content as factors which may affect peroxidation more strongly than resistance shown by α-tocopherol content (Kontush et al., 1994). Nevertheless, our results confirmed by the group of rats fed on diets containing 100% WGO (T4), by contributing extra concentrations of tocopherols in the diets, was found efficient in declining the susceptibility of their lipids to peroxidation in vitro by about 20% as compared to control diets (T0). The results of the present study are also in agreement with the previous findings of Parks et al. (1998) who concluded the reduction in the oxidizability of LDL was significantly associated with the increase in its tocopherol content of diets.

CONCLUSION

The incorporation of WGO in the cookies decreased significantly total cholesterol, LDL and TBARS value however, HDL and triglycerides of rats serum were found to be non-significantly affected by the diets containing WGO.

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