EFFECT OF WALNUT AND SAFFLOWER OIL ON LIPID PROFILE AND GLUCOSE LEVEL IN MALE WISTAR RATS

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Abstract

In the recent decades research on consumption of dietary fats and oil has become an important topic for discussion. This study was carried out to determine the effects soybean (SOBO), walnut (WALO) and safflower (SAFO) oil on lipid profile and glucose level in male Wistar rats. Fifteen male Wistar rats weighting between 150-170 grams were divided randomly into three groups of five rats each. Groups 1 (Control group, SOBO) was assigned to receive 30 grams of the control diet with soybean oil per day. Group 2 was allocated to take 30 grams of the diet with walnut oil per day and group 3 was allocated to receive 30 grams of the diet with safflower oil per day. Statistically insignificant difference was observed between control (SOBO group) and experiments groups (WALO and SAFO) for all growth parameters. *Least value of HDL-C was observed in SOBO group and the highest value was found in WALO group but the difference was insignificant between the groups. TG, LDL-C and VLDL-C was least in SAFO group and lowest value of cholesterol was observed in WALO group but the difference for TC, TG, LDL-C, VLDL-C glucose and insulin was also statistically insignificant the groups. In this study even though insignificant but improvement in blood lipid profiles has been observed without altering other dietary components.*

***Keywords**: safflower, walnut, lipid profile, glucose*

Introduction

Global Burden of Disease Study has reported that CVD is the most important reason for death worldwide (GBD 2016 Causes of Death Collaborators, 2017). In the recent decades research on consumption of dietary fats and oil has become an important topic for discussion (Bester *et al*., 2010). The relationship between saturated and unsaturated fatty acids defines the quality of fats ingested. The higher this ratio is, the greater the amount of unsaturated fatty acids will be and the more their consumption will be advisable. It is noteworthy that monounsaturated fats and polyunsaturated fats do not raise the cholesterol level in the blood and are associated to lesser risks of CVD (Van Elswyk, 1997*)*. Mensink *et al*., (2016) have reported that when PUFAs or MUFAs replaces SFAs than LDL-C decreases which are a strong risk factor for CVD. Pairwise meta-analyses have shown that n-3- and n-6-rich plant oils were more effective in reducing LDL-C and total cholesterol (TC) (Ghobadi *et al.*, 2018). Walnut and safflower are good source of unsaturated fatty acid. Consumption of diet comprising of important phenolic compounds has a remarkable capacity in reducing cholesterol level and platelet aggregation (Shahidi *et al., 2015)*. This study was commenced to evaluate the effects soybean, walnut and safflower oil on lipid profile and glucose level in male Wistar rats.

Material and methods

Experimental design

Animals

The Experimental Animals Center, College of Pharmacy, King Saud University, Saudi Arabia supplied fifteen (150-170 grams) male Wistar rats and the study was performed in accord with research policies of the King Saud University Research Centre. Throughout the experimental period (6 weeks), the rats were kept separately in cages made up of polypropylene at 25 ºC with humidity (50±5%) and 12/12 light / dark cycle. Commercial rodent diet was provided by Grain silos and flour mills, Riyadh, Saudi Arabia.

Diet

The experimental diet (Dytes, USA) was prepared by adding oil to the basal diet by spraying under pressure with continuous mixing during the spraying (Table 1). The fresh chow was weekly mixed with oil to evade oil oxidation. Prepared chow was stored at 4°C until fed. After the adaptation period of a week, the rats were divided into three groups and each group consisted of 5 rats. Each rat received 30 g of diet daily.

Oil

Soybean oil was provided by Dytes, U.S.A and walnut and safflower oil was purchased from the local market. The place of origin of walnut and safflower oil was Turkey.

Assessment of body weight and food consumption

Growth: Growth was noted at the beginning (initial weight) and at the end (final weight) of study in the non-fed state.

Weight gain (g) = final body weight (g)-initial body weight (g)

Growth rate=total weight gain (g)/duration

Biochemical analyses

After six weeks of experimental period the rats were anesthetized with pentobarbital sodium (60 mg/kg body weight). Blood was centrifuged at 3500 rpm for 10 minutes into EDTA Tubes once it was withdrawn from the heart of each rat. After centrifugation the supernatant was separated and stored at -80°C. Total cholesterol, HDL-C, LDL-C, VLDL-C Triglycerides, glucose and insulin kits for the analysis of lipid profile were obtained from Randox Laboratories Ltd. Crumlin, UK.

Statistical analysis

In this study data were expressed as mean ± standard deviation. Data were analyzed using SPSS statistical software package. One way ANOVA at a significance level of $p \leq 0.05$ was used to analyze the differences among the treatment groups and a Post-hoc analysis using Duncan's multiple range tests was performed if the differences were found to be significant.

Results

Effect of different oil (soybean, walnut, safflower) on growth of male Wistar rats

In this study the effect of different oils [soybean (SOBO), walnut (WALO) and safflower (SAFO)] on weight gain and growth rate has been investigated (Fig.1). Initial weight of rats was highest in SAFO group followed by other groups (SAFO > SOBO > WALO). Similar trend was observed in final weight, weight gain and growth rate of rats (SAFO > SOBO > WALO). Statistically insignificant difference was observed between control (SOBO group) and experiments groups (WALO and SAFO) for all growth parameters.

Effect of different oil (soybean, walnut, safflower) on lipid profile and glucose level of male Wistar rats

In table 2 the effect of different oils [soybean (SOBO), walnut (WALO) and safflower (SAFO)] on lipid profile (HDL-C, TC, TG, LDLC, VLDL-C, Glucose and Insulin) has been reported. Least value of HDL-C was observed in SOBO group and the highest value was found in WALO group but the difference was insignificant between the groups. TG, LDL-C and VLDL-C was least in SAFO group and lowest value of cholesterol was observed in WALO group but the difference for TC, TG, LDL-C, VLDL-C glucose and insulin was also statistically insignificant the groups.

Discussion

Nowadays, study on the consumption of dietary fats and oil has turned out to be very significant topic (Bester *et al., 2010)*. Dietary fats and oils provides concentrated source of energy for human metabolic process and are composed of different types of fatty acids such as saturated fatty acid and unsaturated fatty acid which in turn is divided as monounsaturated and polyunsaturated fatty acids (Rezq *et al*., 2010). Diets low in polyunsaturated fatty acids (PUFAs) and rich in saturated fatty acid (SFAs) could cause the development of metabolic abnormalities (Seppanen *et al*., 2013; Sri-Tarino *et al., 2010)*. Numerous studies have been made on the evaluation of dietary FA composition, since any variation in FA composition may cause changes in the lipid composition of cellular structures and lipoprotein synthesis (Ooi *et al*., 2015; Lawrence, *2013*). Rats adjust the food intake depending on the amount of energy (Kcal) consumed (Jean *et al., 2002)*. Like this study Iwamoto *et al*., (2002) also did not noticed any significant difference in growth parameters such as in initial weight, final weight, and weight gain or food efficiency ratio (Iwamoto *et al*., 2002). In this study least value of Glucose level was found in group which consumed walnut oil although the difference between various groups was insignificant. In a previous study also the consumption of walnut oil improved blood glucose level but, no changes were noted for bodyweight in type two diabetic patients (Zibaeenezhad *et al., 2016)*.

A functional food rich in PUFAs and MUFAs are expected to decrease plasma cholesterol levels, and in turn reduces the menace of many chronic diseases including coronary heart disease which is leading cause of death worldwide (Riserus, 2015; Moon *et al.,* 2001). Various studies have found that the type, amount and composition of lipid sources in the diet are decisive factors of the serum lipid profile (Baum *et al., 2012;* Uauy, *2009)*. Regular intake of nuts and seeds are related with encouraging plasma lipid profiles (Luo *et al., 2014;* Bao *et al., 2013;* Mercanligil *et al., 2007)*. In rats the normal values of lipid profile is generally lower than in humans. In rodents, diets high in unsaturated fat were shown to be less adipogenic than diets high in saturated fat, and were attributed to a higher oxidation rate (Leyton *et al., 1987)* and diet-induced thermogenesis (Takeuchi *et al., 1995)*. In this study least value of TG, LDL-C and VLDL-C was observed in SAFO group. Safflower oil is rich in the essential n-6 polyunsaturated fatty acid (PUFA) linoleic acid and n-3 α-linolenic acid and studies have shown its role in the treatment of various diseases such as inflammation, arteriosclerosis, normalizing menstruation, promoting blood coagulation (Zhao *et al., 2009)*. The alpha linolenic acid (ALA) content of SAFO can decrease TG concentration through the inhibition of hepatic VLDL-TG synthesis and secretion that is secondary to a decrease in TG synthesis. This decrease in VLDL-TG secretion may be due to the decrease in the expression of hepatic gene transcription factor sterol regulatory element binding proteins (SREBP-1c) which is the key switch in controlling lipogenesis, (Asp *et al*., 2011). In this study even though insignificant but improvement in blood lipid profiles has been observed without altering other dietary components with relatively normal blood lipid profiles at baseline. As compared to SOBO the concentration of HDL was more in both WALO and SAFO which might be due to the presence of omega-3 fatty acid (Arterburn *et al.,* 2008). Various Epidemiological studies have suggested that ingesting α linolenic acid might be useful to prevent humans from cardiac diseases (de Lorgeri *et al., 1999;* Ascherio *et al*., 1996). Linoleic acid (LA) acts as a ligand of PPAR transcription factors and inhibits transcription of certain gene-encoding enzymes involved in the synthesis of hepatic lipogenesis, and instead stimulates the synthesis of enzymes involved in β-oxidation (Nagao *et al*., 2008). Even though as compared with diets high in PUFAs the cardioprotective effect of diets high in MUFAs is controversial, it is commonly accepted that the substitution of SFAs and trans fatty acids with MUFAs or PUFAs is advantageous for cardiovascular health (de Lorgeri *et al.,* 1999*;* Marai and Massalha, 2014*)*.

Conclusion

In this study even though insignificant but improvement in blood lipid profiles has been observed without altering other dietary components The reason behind the positive effect on lipid profile could mainly be due to the lipid content of safflower oil and walnut.

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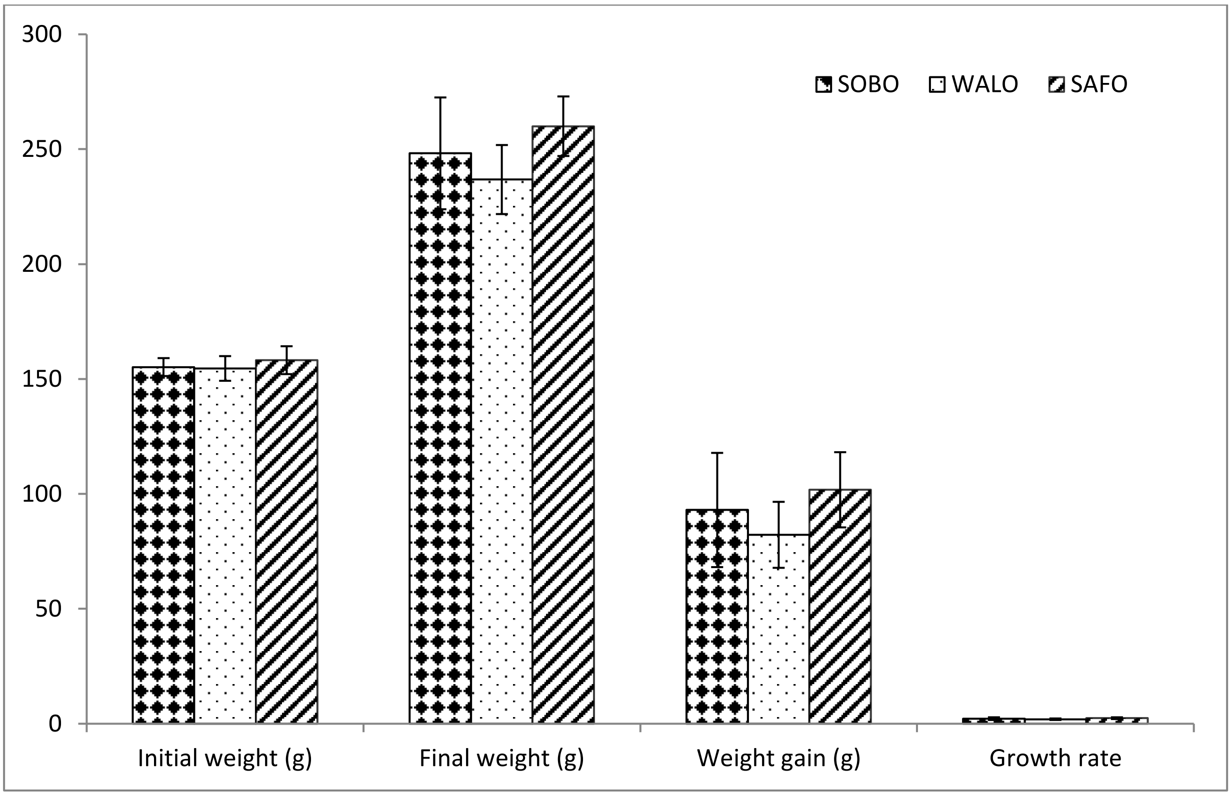
Table 1: Formulation of the AIN - 93M diet for maintenance of rats

Ingredients	**SOBO Group (g/kg diet)**	**WALO Group (g/kg diet)**	**SAFO Group (g/kg diet)**
Corn starch	456.6	456.6	456.6
Casein	140	140	140
Maltodextrin	145	145	145
Sucrose	100	100	100
Cellulose	50	50	50
Mineral MIX (AIN 93-- MX)	35	35	35
Vitamin mix (AIN 93 VX)	10	10	10
L -Cystine	1.8	1.8	1.8
Choline	2.5	2.5	2.5
Tertiary Butylhydroqyinone (TBHQ)	8	8	8
Soybean oil	50	0	0
Walnut oil	0	50	0
Safflower oil	0	0	50

Table 2: Effect of different oil (soybean oil, walnut oil, safflower oil) on lipid profile and glucose level of male Wistar rats

	SOBO	WALO	SAFO
HDL-C (mg/dl)	39.55 ± 10.04^{a}	60.49 ± 18.38^{a}	57.91 ± 25.22^{a}
TG (mg/dl)	102.86 ± 3.88^{a}	102.86 ± 3.71^{a}	99.09 ± 4.10^{a}
TC (mg/dl)	74.32 ± 15.38^{a}	55.20 ± 10.20^{a}	65.54 ± 15.77^{a}
LDL-C (mg/dl)	33.24 ± 17.77^{a}	33.28 ± 17.79^{a}	32.21 ± 17.09^{a}
VLDL-C (mg/dl)	20.57 ± 0.78^{a}	20.57 ± 0.74^{a}	19.82 ± 0.82^{a}
Glucose (mg/dl)	86.47 ± 11.92^{a}	77.88 ± 12.74^{a}	89.78 ± 11.98^{a}
Insulin (mmol/L)	5.44 ± 0.63^{a}	4.78 ± 0.77^{a}	5.08 ± 1.58^{a}

Where SOBO: soya bean oil; WALO: walnut oil; SAFO: safflower oil. Data are expressed as the mean ± standard deviation; Model ANOVA, p values < 0.05 are significant. Different superscript indicates significant differences among various groups as indicated by ANOVA followed by Duncan's multiple range



Graph 1: Growth indicators of male Wistar rats fed with different oil

Where SOBO: soya bean oil; WALO: walnut oil; SAFO: safflower oil. Data are expressed as the mean ± standard deviation; Model ANOVA