International Journal of Education and Social Science Research

ISSN 2581-5148

Vol. 2, No. 04; 2019

THE EFFECT OF ORAL SOY FLOUR ON REPAIR OF LIPID PROFILE ON WISTAR WHITE RATS STICK WHICH HAS DYSLIPIDEMIA

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ABSTRACT

Dyslipidemia is a condition in which a component lipid profile increases or decreases from normal limit in the blood. Lipid profile is an indicator to determine the occurrence of Dyslipidemia, one of the risk factor for cardiovascular disease is Dyslipidemia. The prevention can be with a low-calorie diet and followed by consuming soy flour which is rich in Isoflavones and Lechitins. Determine the effect of soybean flour on the improvement of lipid profile (HDL and LDL) wistar strain white rats that have Dyslipidemia. This study was conducted experimentally, with Pre-test and Post-test Control Group Design. This study was conducted for 38 days. The sample of this study was 24 wistar male-female white rats, aged 12-16 weeks and having a weight of + 200 grams that experienced Dyslipidemia which was divided into 4 groups, the control group only received high-fat feed only while the treatment group was given high-fat feed, and soy flour with doses of 0.3 mg, 0.6 mg and 0.9 mg each per administration three times a day starting from 23-37 days. A high-fat diet is given starting on days 8-22. The treatment effect test uses One Way Anova, followed by LSD (Least Significant Difference test) and t-paired test. The results of the t-paired test showed a significant decrease in LDL levels (p = 0.012) and there was a significant increase in HDL levels (p = 0.037) after the administration of soy flour in the group that received a dose of 0.9 mg / dl / times (p = 0.012) and while in other doses there is no significant decrease in LDL and increase in HDL. One-Way ANOVA test between treatment groups showed significant differences in LDL cholesterol levels (p = 0.001) and HDL (p = 0.001), for the Least Significant Difference test only the treatment group with a dose of 0.9mg / dl / times indicating significant differences with other groups (p = 0,000) (p = (0,006) (p = 0,024). It was concluded that the administration of soy flour can reduce LDL cholesterol levels and increase HDL cholesterol levels in high-fat diet-induced rats at a dose of 0.9 mg / dl / times 3 times a day.

KEYWORDS: soy flour, white rat dyslipidemia, lipid profile

INTRODUCTION

Dyslipidemia is a decrease in High Density Lipoprotein (HDL) cholesterol or an increase in cholesterol, triacylglycerol in the blood. This situation contributes to the occurrence of atherosclerosis as one of the causes of coronary heart disease, peripheral arterial disease, and cerebrovascular disease (as a non-infection disease) which causes the highest mortality in the whole world. The WHO (World Health Organization) said that in 2008 there were 17.3 million people dying of cardiovascular disease and in the year 2030 it will occur more than 23 million people will die from disease.

ISSN 2581-5148

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The above diseases need comprehensive and conventional handling. Comprehensive use of drugs that have often been used in health services, while components can also be used but are not yet familiar with health services. One of them is the provision of soybeans, which is one source of vegetable protein which can reduce total cholesterol, low density lipoprotein (LDL) and triglycerides, and can increase HDL. This isoflavone and Lechitins content can function to prevent and repair dyslipidemia which contributes to the occurrence of arteriosclerosis.

Isoflavones can improve the condition of dyslipidemia through the process of suppressing adipogenesis. Isoflavones in the human body can stimulate the formation of HDL cholesterol in the liver and significantly reduce LDL cholesterol and Triacylglycerol Isoflavones also work to prevent the accumulation of fat in the body by inhibiting the work of lipogenic enzymes that regulate lipid uptake, namely the enzyme lipoprotein lipase (LPL) if the activity of this enzyme is lowered, the fat deposits in adipocyte cells will decrease. Isoflavones also provide a negative stimulus to the hungry center in the hypothalamus so that it decreases appetite.

Lechitins have the effect of increasing the work of the cholesterol HMG coa-reductase and 7 alpha hydroxylase enzymes which allows to increase bile acid production and bile acid secretion, resulting in a decrease in cholesterol because it is used to produce bile acids, especially the concentration of LDL cholesterol.

2. METHODS

The type of research used in this study was analytic research with pure experimental pretest-posttest control group design, which consisted of 4 experimental animal groups as research subjects, namely 1 control group and 3 treatment groups. This research was conducted at the Try Animal Laboratory of the Faculty of Medicine, Wijaya Kusuma University, Surabaya and was conducted for 38 days.
The research population that will be maintained by the researchers is male wistar strain white rats aged 12-16 weeks, as many as 30 rats, with a body weight of + 200 grams. The sample size in each study group was determined by the formula Fraenkle and Wallen and the results of the sample size were ≥ 5 each group, according to the results the researchers used 6 individuals per group. The sample used was taken from a population of 30 rats then randomly selected 24 Wistar strain white rats weighing + 200 grams to be used as research samples and 6 more were used to reserve samples. After that the rats were adapted for 7 days to avoid stress and diseases suffered by rats, then on the 8th day the sample rats were given a diet high in saturated fat until the 37th day to get rats with dyslipidemia.

Where in this study all research groups were made to experience dyslipidemia before treatment by providing a high saturated fat diet of 15 grams a day with the composition: Comfeed BR-1 55%, 28% wheat flour, 6% yolk, 0.2% Kolat Acid, 10% goat oil, 1% coconut oil and sufficient water16.17 In the treatment group soybean flour was also given orally from day 23-37 (flour diluted to 4ml then pressed to mouth of rats) at different doses in each group treatment group 1 (0 , 3 mg), treatment 2

(0.6 mg), treatment 3 (0.9 mg) this dose is based on the amount of isoflavone recommended in humans is 30-100 mg per day and rat stomach volume. 17, 18

In this study the cholesterol level examination method and the calculation of HDL-LDL levels used the examination method of the Bioassay system with the Colometric Procedure and the results were expressed in mg / dl.19 Results of examination of LDL cholesterol and HDL serum levels of rats before and after treatment in each group then analyzed statistically. The first test conducted was the normality test of the data using Saphiro-Wilk test, then the homogeneity test with the Levene test followed by a paired sample t-test to determine the mean differences before and after treatment. The mean differences between groups before and after giving soy flour, were analyzed by the One-Way ANOVA test followed by the treatment effect test using LSD (Least Significant Difference test).

RESULTS

Data Normality Test

LDL and HDL data before and after treatment in each group tested their normality using the Saphiro-Wilk test. The results show that the data are normally distributed ($p > 0.05$),

Test Homogeneity of Data between Groups

LDL and HDL data between groups before and after treatment were tested for homogeneity using the Leven's test. The results show homogeneous data ($p > 0.05$).

Comparability Test Before Treatment

The Comparability Test aims to compare the mean LDL and HDL between groups before being given treatment. The results of the significance analysis by the One Way Anova test are presented in Table 1 and Table 2

Table 1.
Mean of LDL Between Groups Before Treatment

Subject Group	n		Mean LDL (mg/dl)	Std. deviation	F	P
Control	6		64.208	10.181	0.368	0.777
Treatment 1	6		60.382	11.235		
Treatment 2	6		58.197	9.259		
Treatment 3	6		59.291	11.424		

Table 1 above shows that the mean of LDL of the control group was 64,208 ± 10.181, the average treatment group 1 was 60,382 ± 11,235, the mean treatment group 2 was 58,197 ± 9.259 the mean of treatment group 3 was 59,291 ± 11,424. Significant analysis with One Way Anova test shows that the value of F = 0.368 p value = 0.777. This means that the mean LDL pre-test in the four groups was not significantly different ($p > 0.05$).

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Table 2.
Mean of HDL Between Groups Before Treatment

Subject Group	n		Mean HDL (mg/dl)	Std. deviation	F	P
Control	6		22.678	6.592	0.485	0.696
Treatment 1	6		21.859	8.716		
Treatment 2	6		25.683	6.278		
Treatment 3	6		25.956	7.435		

Table 2 above shows that the mean HDL of the control group was 22,678 ± 6,592, the mean treatment group 1 was 21,859 ± 8,716, the mean treatment group 2 was 25,683 ± 6,278. The mean treatment group 3 was 25,956 ± 7,435. Significance analysis using the One Way Anova test shows that the value of F = 0.485 p value = 0.696. This means that the mean HDL pre test in the four groups was not significantly different (p> 0.05).

Treatment Effect Test

The treatment effect test aims to compare the mean LDL and HDL between groups after treatment. The results of the significance analysis by the One Way Anova test are presented in Table 3 and Table 4 below.

Table 3.
Mean of LDL Between Groups After Treatment

	Subject Group	n		Mean LDL (mg/dl)	Std. deviation	F	P
	Control	6		71.311	7.947	12.522	0.001
	Treatment 1	6		51.639	10.611		
	Treatment 2	6		49.727	10.758		
	Treatment 3	6		37.978	8.638		

Table 3 above shows that the mean LDL of the control group was 71.311 ± 7.947, the mean treatment group 1 was 51.639 ± 10.611, the mean treatment group 2 was 49,727 ± 10,758. The mean treatment group 3 was 37,978 ± 8,638. Significance analysis with One Way Anova test shows that the value of F = 12,522 p = 0.001. This means that the mean LDL post test in the four groups was significantly different (p> 0.05).

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Table 4.
Mean of HDL Between Groups AFTER Treatment

Subject Group	n		Mean HDL (mg/dl)	Std. deviation	F	P
Control	6		18.852	2.694	9.455	0.001
Treatment 1	6		27.869	6.557		
Treatment 2	6		30.328	9.657		
Treatment 3	6		40.164	7.165		

Table 4 above shows that the mean HDL of the control group was 18,852 ± 2,694, the mean treatment group 1 was 27,869 ± 6,557, the mean treatment group 2 was 30,328 ± 9,657. The average treatment group 3 was 40,164 ± 7,165. The significance analysis using the One Way Anova test shows that the F value of 9.455 p = 0.001. This means that the mean HDL post-test in the four groups was significantly different (p> 0.05).

To find out the mean LDL and HDL of different groups with the control group, further tests were needed with the Least Significant Difference test (LSD). The results of Post Hoc analysis are presented in Tables 5 and 6 below.

Table 5.
Differences in mean of LDL levels between groups after treatment

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Sig.	Description
Post LDL	Control	Treatment 1	19.672000*	0.002	Significantly Different
		Treatment 2	21.584333*	0.001	Significantly Different
		Treatment 3	33.333000*	0.000	Significantly Different
	Treatment 1	Control	-19.672000*	0.002	Significantly Different
		Treatment 2	1.912333	0.733	Not Sig. Different
		Treatment 3	13.661000*	0.022	Significantly Different
	Treatment 2	kontrol	-21.584333*	0.000	Significantly Different
		Treatment 1	-1.912333	0.733	Not Sig. Different
		Treatment 3	11.748667*	0.046	Significantly Different
	Treatment 3	kontrol	-33.333000*	0.001	Significantly Different
		Treatment 1	-13.661000*	0.022	Significantly Different
		Treatment 2	-11.748667*	0.046	Significantly Different

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Table 6. Differences in mean of HDL levels between groups after treatment

Dependent Variable	(I) perlakuan	(J) perlakuan	Mean Difference (I-J)	Sig.	Keterangan
Post HDL	Control	Treatment 1	-9.016667*	0.037	Significantly Different
		Treatment 2	-11.475667*	0.010	Significantly Different
		Treatment 3	-21.311667*	0.000	Significantly Different
	Treatment 1	Control	9.016667*	0.037	Significantly Different
		Treatment 2	-2.459000	0.549	Not Sig. Different
		Treatment 3	-12.295000*	0.006	Significantly Different
	Treatment 2	kontrol	11.475667*	0.010	Significantly Different
		Treatment 1	2.459000	0.549	Not Sig. Different
		Treatment 3	-9.836000*	0.024	Significantly Different
	Treatment 3	kontrol	21.311667*	0.000	Significantly Different
		Treatment 1	12.295000*	0.006	Significantly Different
		Treatment 2	9.836000*	0.024	Significantly Different

Based on the results of follow-up tests (Post Hoc Test) with the Least Significant Difference-test (LSD) test above can be described as follows.

1) The mean of LDL of the control group was significantly different from the treatment group 1, treatment 2 and treatment 3.
2) The mean of LDL treatment group 1 was significantly different from the control group, treatment 3 and treatment 2 was not significantly different
3) The mean of LDL treatment group 2 was significantly different from the control group, treatment 3 and not significantly different from the treatment group 1.
4) The mean of LDL of treatment group 3 was significantly different from the control group, treatment group 1 and treatment 2
5) The mean of HDL of the positive control group was significantly different from the treatment group 1, treatment 2 and treatment 3
6) The mean of HDL treatment group 1 was significantly different from the control group and treatment 3, not significantly different from treatment 2
7) The mean of HDL treatment group 2 was significantly different from the control group, treatment 3 and different were not treated with treatment 3.
8) The mean of HDL in treatment group 3 was significantly different from the control group, treatment 1 and treatment 2

The Difference between LDL and HDL before and after treatment

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To find out the difference between LDL and HDL between before and after treatment was analyzed by t-paired test. The results of the analysis are presented in Tables 7 and 8 below.

Table 7.
The Difference of LDL before and after treatment

Group	Before	After	Mean	Description	p	Description
Control	64.208	71.3113	-7.104	Increase	0.216	Not. Sig. Different
Treatment 1	60.382	51.6393	8.743	Decrease	0.129	Not. Sig. Different
Treatment 2	58.197	49.7270	8.471	Decrease	0.254	Not. Sig. Different
Treatment 3	59.291	37.9783	21.312	Decrease	0.012	Significantly Different

Based on the results of the t-paired test, it was found that in the control group there was an increase in LDL but not significant (p> 0.05) then in the group treatment 1, treatment 2 decreased LDL but not significant (p> 0.05) and treatment 3 there was a significant decrease in LDL (p <0.05).

Tabel 8.
The Difference of LDL before and after treatment

Group	Before	After	Mean	Description	p	Description
Control	22.678	18.852	3.825	Increase	0.328	Not. Sig. Different
Treatment 1	21.858	27.869	-6.011	Decrease	0.276	Not. Sig. Different
Treatment 2	25.683	30.328	-4.645	Decrease	0.482	Not. Sig. Different
Treatment 3	25.956	40.164	-14.207	Decrease	0.037	Significantly Different

Based on the results of the t-paired test it was found that in the control group there was a decrease in HDL but not significant (p> 0.05), while in the treatment group 1 and treatment 2 there was an increase in HDL but not significant (p> 0.05) then treatment group 3 there was a significant increase in HDL (p <0.05).

DISCUSSION

Induction of dyslipidemia was successful in all groups because the mean LDL level was > 27 mg / dl, and the mean HDL level was <35 mg / dl. In accordance with tables 1 and 2, there were no

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significant differences from the mean LDL and HDL levels of pre-test in each group. This indicated that the induction of dyslipidemia was successful and there were no differences in the results of the significant induction of dyslipidemia in each group. Giving soy flour orally in the treatment group according to tables 3 and 4 shows a significant difference in mean LDL and HDL in each group, and according to tables 5 and 6 only the treatment group 3 had a mean HDL and LDL different from the other groups. Likewise in tables 7 and 8, the control group experienced an increase in LDL mean and HDL decrease that was not significant, then in the treatment groups 1 and 2 there was a decrease in LDL and an increase in HDL that was not significant, only in group 3 which decreased LDL and HDL.

The decrease in LDL and increase in HDL in treatment groups 1, 2, and 3 can be caused by the content of Isoflavones and Lechitins in soy flour. Isoflavones in the body can stimulate the formation of HDL cholesterol in the liver and reduce LDL cholesterol and Triacylglycerol. Isoflavones can improve the condition of dyslipidemia through the process of suppressing adipogenesis. Isoflavones also work to prevent the accumulation of fat in the body by inhibiting the work of lipogenic enzymes that regulate lipid uptake, the enzyme lipoprotein lipase (LPL). When this enzyme activity is lowered, the fat deposits in adipocyte cells will decrease.
Lechitins in the liver help metabolize fat with polyunsaturated fat in it, lecithine will help the process of absorption of fat in the liver. The workings of Lecithine in helping fat metabolism through its ability to improve its metabolism.

Lechitins have the effect of increasing the work of the cholesterol HMG coa-reductase and 7 alpha hydroxylase enzymes which allows to increase bile acid production and bile acid secretion, resulting in a decrease in cholesterol because it is used to produce bile acids, especially the concentration of LDL cholesterol. Lechitins are also known to stimulate the formation of HDL cholesterol in the liver. Therefore, in the treatment group there was a decrease in LDL and an increase in HDL levels whereas in the control group that was not given soy flour there was an increase in LDL and a decrease in HDL was caused by the absence of the effect of giving soy flour as in treatment groups 1, 2 and 3. But from the results the analysis carried out was only the treatment group 3 which experienced a decrease in LDL and a significant increase in HDL while the treatment groups 1 and 2 only experienced a decrease in LDL and an increase in HDL in a non-significant way. This is because the doses in treatment groups 1 and 2 are not optimal for achieving meaningful results.

CONCLUSION

Based on the results of this study it can be concluded that soy flour given orally can reduce LDL levels and can increase HDL levels in the body of rats that experience dyslipidemia so that it can improve the levels of mouse lipid profiles due to the content of Isoflavones and Lechitins. A significant dose of lowering LDL levels and increasing HDL levels is 0.9 mg / head / times given 3 times a day.

REFERENCES

1. Z˘eljko Reiner, Alberico L. Catapano, Guy De Backer, Ian Graham, Marja-Riitta Taskinen, Olov Wiklund, Stefan Agewall , Eduardo Alegria, M. John Chapman, Paul Durrington, Serap Erdine, Julian Halcox, Richard Hob, John Kjekshus, Pasquale Perrone Filardi, Gabriele Riccardi, Robert F. Storey, David Wood. *ESC/EAS Guideline For The management of Dyslipidemias*. European Heart Journal (2011) 32, 1769–1818 doi:10.1093/eurheartj/ehr158

2. James Stein, Jessica Bartell, Fred Brodsky, Maggie Dugan, Lawrence Fleming, Derek Hubbard, Pam Kittleson, Jennifer Schauer. 2008. *Guidelines for the Diagnosis and Management of Dyslipidemias for Adults > 18 Years Old* - February 2008

3. Chujit W, Thaksin P, Dhatsuwan J, dan Kaewtwm Y. 2008. *Waist Circumference is the best predictor of Dyslipidemia among Thai People : A Cross-Sectional Study*. Makalah disajikan dalam international conference " Health and the Changing World, 11-13 November 2008

4. Giray Kabakci, Nevres Koylan, Baris Ilerigelen, Omer Kozan, Kemalettin Buyukozturk, 2008, *Impact of dyslipidemia on cardiovascular risk stratification of hypertensive patients and association of lipid profile with other cardiovascular risk factors: results from the ICEBERG study*, Integrated Blood Pressure Control 2008:1 5–13

5. WHO. Cardiovaskuler disease. 2008. http://www.who.inte/cardiobaskuler_disease/en

6. Dalimartha S, 2008. 36 *Resep Tumbuhan Obat untuk menurunkan Kolesterol*. Jakarta : Penebar Swadaya.

7. Paul Nestel, 1995, *Role of Soy Protein in Cholesterol-Lowering How Good Is It?*, Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org DOI: 10.1161/01.ATV.0000035520.25551.97

8. Khomsan, A. 2006. *Solusi Makanan Sehat.* Jakarta: PT Rajagrafindo Persada

9. Amouni Mohamed Mourad, Eder de Carvalho Pincinato, Priscila Gava Mazzola, Maricene Sabha, Patricia Moriel, 2009, *Clinical Study Influence of Soy Lecithin Administration on Hypercholesterolemia*, Hindawi Publishing Corporation Cholesterol Volume 2010, Article ID 824813, 4 pages doi:10.1155/2010/824813

10. Naaz A[1], Yellayi S, Zakroczymski MA, Bunick D, Doerge DR, Lubahn DB, Helferich WG, Cooke PS. 2003. *The soy isoflavone genistein decreases adipose deposition in mice.* US National Library of Medicine National Institutes of Health. Endocrinology. 2003 Aug;144(8):3315-20.

11. Siyan Zhan and Suzanne C HO, 2005. *Meta –analysis of the effect of soy protein containing isoflavones on the lipid profile,*. American Society for Clinical Nutrition.

12. Cooke Paul S, Afia Naaz, 2004, *Role of estrogen in Adipocyte Development and function, Society of experimental biology and medicine*, Vol 1, 1535-3702/04/22911-1127

13. M,-J. LeBlanc, S. Brunet, G. Bouchard, et al., *Effects of dietary soybean Lechitin on plasma lipid transport and hepatic cholesterol metabolim in rats*, Journal of Nutritional Biochemistry, Vol. 14, no 1, pp. 40-48, 2003.

14. Amouni Mohamed Mourad, Eder de Carvalho Pincinato, Priscila Gava Mazzola, Maricene Sabha, Patricia Moriel, 2009, *Clinical Study Influence of Soy Lecithin Administration on Hypercholesterolemia,* Hindawi Publishing Corporation Cholesterol Volume 2010, Article ID 824813, 4 pages doi:10.1155/2010/824813

15. Pocock, SJ. 2008. *Clinical Trials A Practical Approach.* England: John Wiley & Son.

16. Triana, E. dan N. Nurhidayat. 2006. *Pengaruh pemberian beras yang difermentasi oleh Monascus Purpureus terhadap darah tikus putih (Rattus Sp.) hiperkolesterolemia*. J.Biodiversitas 7(4): 317-321

17. Bustaman, N. 2008. *Fitoestrogen dan kesehatan Tulang.*Volume 19 nomor 3, 146-150. Bina Widya

18. George Krinke. 2000. *The Laboratory Rat1s t Editio. USA.* Academic Press.

19. BioAssay System. 2012. *EnzyChromTM AF HDL and LDL/VLDL Assay Kit (E2HL-100)* Quantitative Colorimetric/Fluorimetric Determination of HDL and LDL/VLDL. Hayward, CA 94545, USA ·

20. Manuel T. Velasquez1 and Sam J. Bhathena. 2007. *Role of Dietary Soy Protein in Obesity. International Journal of Medical Sciences*. ISSN 1449-1907 www.medsci.org 2007 4(2):72-82

21. Y. Lin, G. W. Meijer, M. A. Vermeer, and E. A. Trautwein, 2004.*Soy protein enhances the cholesterol-lowering effect of plant sterol eaters in cholesterol-fed harmsters*, Journal of nutrit ion vol. 134, no 1,pp. 143-148