

Job's Tears Ameliorated the Lipid Profile of Diabetic Rats

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Abstract

The purpose of this study was to investigate the potential hypolipidemic effect of job's tears diet with the controlled intake of fiber in streptozotocin induced diabetic rats. Forty eight rats were fed either control diet or experimental diets(Raw, milled job's tears(RMJ); Raw, whole grain job's tears(RWJ); Steamed, milled job's tears(SMJ); Roasted, milled job's tears(OMJ)) for 3 weeks. The levels of total cholesterol(TC), low density lipoprotein cholesterol(LDL-C), high density lipoprotein cholesterol(HDL-C), free fatty acid(FFA) and triglyceride(TG) in plasma, liver and skeletal muscle were compared. Compared to diabetic control group, the level of plasma TC was lower in OMJ and SMJ fed rats, and was not different from that in RMJ and RWJ fed rats. The level of LDL-C was significantly lower in RMJ, OMJ and SMJ fed rats. For plasma HDL-C levels, no difference was observed among all group. Plasma FFA level was significantly lower in RMJ, SMJ and RWJ fed rats. Plasma TG levels of all job's tears fed groups were 50~70% lower and liver TG levels of all job's tears fed groups were 70~80% lower than those of diabetic control group. For muscle TG, no differences were seen among all groups. These results suggest that steamed job's tears and roasted job's tears could have the potential on improving lipid profile of diabetes in clinical setting and the different cooking methods of job's tears might affect the lipid profile of the diabetic rats.

Key words: job's tears, lipid profile, hypolipidemic food, diabetic rats

INTRODUCTION

Job's tears(*Coix Lachryma-Jobi*) has been used as a diuretic, stomachic, analgesic, antispasmodic and hypoglycemic agent in oriental folk medicine without scientific verification. Recently, physiologically active substances possessing antiphlogistic, anti-tumor promoting activity have been isolated(1-3). But no active constituent related to the treatment for diabetes other than dietary fiber from job's tears has been isolated. Studies in both humans and animals support, in general, the view that the use of diets high in complex carbohydrates including high level of dietary fiber has been reported to normalize blood glucose and lipid levels in individuals who are diabetic or hyperlipidemic(4-8). Because the dietary fiber content of job's tears is not higher or even lower than other grains such as barley, millet or sorghum(9,10) and job's tears is not an economically favorable grain, it is important to determine whether job's tears functions as therapeutic agents on diabetes. Previously, we have reported that the hypoglycemic effect of job's tears diet is not significant when the amount of total fiber consumption is controlled (11). Although the hypoglycemic activity of job's tears *per*

se was not significant, if it demonstrates that job's tears diet improves the lipid profile which has been postulated as a useful indicator of complications in diabetes, it is worthy to stick on job's tears diet. Especially, the digestibility of starchy food is affected by the cooking methods (12,13) and this digestibility can affect the level of blood lipid. Thus, as a part of our continuing studies on biological activities of natural products, we have investigated the potential curative activities of job's tears with different cooking methods on diabetes.

MATERIALS AND METHODS

Animals and diets

Forty eight male Sprague-Dawley rats of 160~180g were divided into 6 groups; normal group, diabetic-control group, diabetic-raw, milled job's tears group(RMJ), diabetic-raw, whole grain job's tears group(RWJ), diabetic-steamed milled job's tears group(SMJ), diabetic-roasted milled job's tears group(OMJ). Diabetes were induced by streptozotocin injection into the tail vein(45mg/kg body weight), which affects the β -cell of pancreas specifically

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(14) prior to initiating the experimental diets. Diabetic rats were confirmed with blood glucose level 24 hour after streptozotocin injection. Animals received 50% job's tears diet or control diet for 3 weeks. Control diet was a vitamin-free casein based semisynthetic diet which met AIN-76 recommendation(15,16). The nutritional composition of 50% job's tears diet was manipulated to get the similar composition of the control diet(Table 1). The job's tears was provided by Yeonchon Agricultural Cooperative Association. Job's tears was roasted at 100°C for 30 minutes for roasted job's tears and steamed at 100°C for 1 hour for steamed job's tears. Raw, roasted, steamed, and whole grain job's tears were dried and powdered. Prior to initiating the experimental diets, rats were given ad libitum access to the control diet for 1 week to adapt to the diet and feeding schedule and to bring all the rats to a similar metabolic status. All animals were weighed weekly and the food intake was measured daily.

Sample collection

Blood was drawn from eye vein in postprandial state at 9~10 a.m. every week. Animals were anesthetized with ether and sacrificed by decapitation. Immediately following decapitation, blood was collected in heparinized tubes and centrifuged to separate the plasma. Organs were rapidly blotted dry and weighed. Plasma and tissues were stored at -70°C until analyzed.

Analysis

Plasma total cholesterol(TC) was analyzed with commercial kit based on enzymatic method(Youngdong Pharmaceutical Co, Korea). HDL-cholesterol(HDL-C) was analyzed with commercial kit based on same analytical method as total cholesterol, after the precipitation of LDL,

VLDL and chylomicron with polyethyleneglycol(International Reagent Co., Japan). Atherosclerotic index was calculated as (TC-HDL-C)/HDL-C. Triglyceride(TG) was analyzed with commercial kit based on Trinder method (Youngdong Pharmaceutical Co., Korea). Free fatty acid (FFA) was analyzed with commercial kit utilizing Acyl CoA synthetase-Acyl CoA oxidase(NEFAZYME-S, Eiken Chemical Co., Japan). Tissue samples were homogenized in cold sodium phosphate buffer(0.02M, pH 7.0). Aliquots of the tissue homogenates were analyzed as the same method as that of plasma. For statistical analysis, all data were first evaluated by analysis of variance. For those F values which were significant, the least significant difference test was performed. P value <0.05 was considered to be statistically significant.

RESULTS

At various points during the study, the diabetic status of the rats was evaluated using plasma glucose level, total body weight gain and feed efficiency ratio. Compared to normal group, diabetic group showed higher plasma glucose(142±36 vs 592±287mg/100ml) throughout the study. The total body weight gain(BWG) and feed efficiency ratio(FER) of diabetic rats were significantly lower than those of normal rats(BWG: 91±11 vs 31±17g, FER: 0.270±0.040 vs 0.076±0.080). The effect of job's tears diet on the change of plasma cholesterol level in rats is shown in Fig. 1. The plasma total cholesterol level of RMJ and RWJ was not different from that of diabetic control group and higher than that of normal group throughout the study. After the second week of job's tears diet, the plasma total cholesterol level of OMJ tended to be decreased and significantly lower than that of diabetic control group at the third week of job's tears diet. The plasma total cholesterol level of SMJ was significantly

Table 1. Composition of diet(%)

| | Control diet | Job's tears diet Raw, Roasted, Steamed | Job's tears diet Whole grain |
|----------------------|--------------|---|---------------------------------|
| Casein(vitamin-free) | 20.00 | 12.65 | 12.81 |
| DL-methionine | 0.30 | 0.30 | 0.30 |
| Sucrose | 50.00 | 24.96 | 29.00 |
| AIN. vitamin mixture | 1.00 | 1.00 | 1.00 |
| AIN mineral mixture | 3.50 | 3.25 | 2.43 |
| Cellulose | 5.00 | 3.65 | 3.07 |
| Corn oil | 5.00 | 3.99 | 1.19 |
| Choline bitartrate | 0.20 | 0.20 | 0.20 |
| Corn starch | 15.00 | — | — |
| job's tears | — | 50.00 | 50.00 |

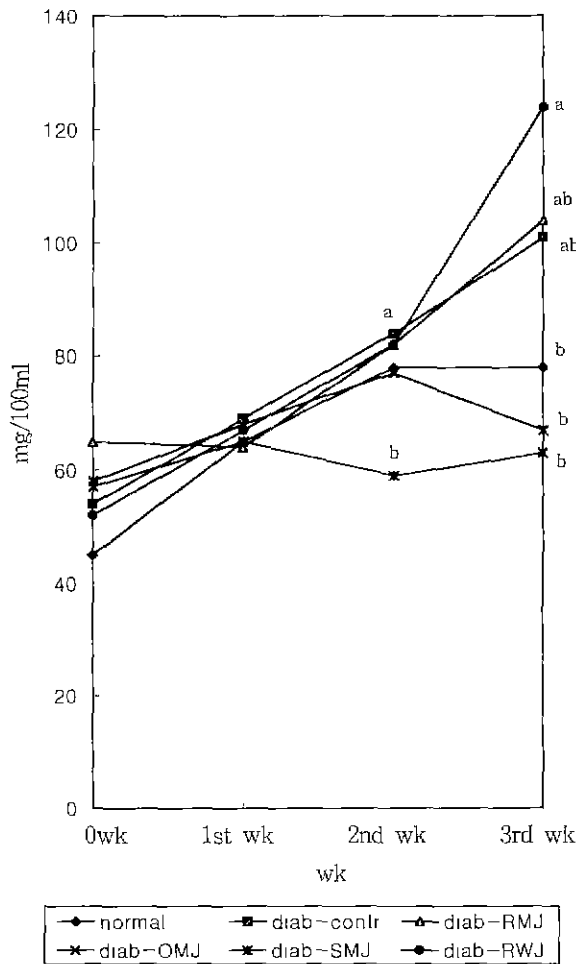


Fig. 1. The effect of job's tears on the change of plasma cholesterol in rats.

Within a given column, those values with different letters are significantly different at $p < 0.05$: RMJ=raw, milled job's tears. OMJ=roasted, milled job's tears: SMJ=steamed, milled job's tears: RWJ=raw, whole grain job's tears.

lower than that of diabetic control group at the second week and this tendency was kept until the third week. Table 2 shows the effect of job's tears diet on the plasma cholesterol profile in rats. Plasma total cholesterol level of OMJ and SMJ tended to be lower than that of diabetic control group although this difference was not significant due to the large standard deviation, and was close to that of normal group. Compared to diabetic control group, the level of plasma total cholesterol was not different in RMJ and RWJ. For plasma HDL-C levels, no difference was seen among all group. Compared to diabetic control group, arteriosclerotic index decreased significantly in SMJ, tended to be decreased in OMJ and did not decrease in RMJ and RWJ. Table 3 shows the effect of job's tears diet on the level of free fatty acid and triglyceride in plasma and tissues of rats. Compared to

Table 2. The effect of job's tears on plasma cholesterol profile in rats¹⁾

| | TC ²⁾ (mg/100ml) | HDL-C (mg/100ml) | Atheroscle- rotic Index |
|--------------------|--------------------------------|----------------------------|----------------------------|
| Normal | 78.11 ± 14.44 ^{bc)} | 38.99 ± 11.18 ^a | 1.00 ± 0.20 ^b |
| Diabetic-control | 101.13 ± 25.02 ^{ab} | 40.96 ± 13.12 ^a | 1.46 ± 0.37 ^{ab} |
| -RMJ ⁴⁾ | 104.44 ± 33.62 ^{ab} | 40.89 ± 8.02 ^a | 1.55 ± 0.57 ^{ab} |
| -OMJ | 67.85 ± 43.60 ^b | 32.83 ± 9.62 ^a | 1.06 ± 0.64 ^b |
| -SMJ | 63.80 ± 8.48 ^b | 42.85 ± 9.15 ^a | 0.48 ± 0.07 ^c |
| -RWJ | 124.43 ± 31.08 ^a | 44.26 ± 8.58 ^a | 1.81 ± 0.45 ^a |

¹⁾Values are mean ± SEM, n=8

²⁾TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol,

³⁾Within a given column, those values with different superscripts are significantly different at $p < 0.05$

⁴⁾RMJ: Raw, milled job's tears, OMJ: Roasted, milled job's tears. SMJ: Steamed, milled job's tears, RWJ: Raw, whole grain job's tears

Table 3. The effect of job's tears on the level of free fatty acid and triglyceride of plasma and tissues in rats¹⁾

| | Plasma | | Liver | Muscle |
|--------------------|---------------------------------|----------------------------|---------------------------|-------------------------|
| | FFA ²⁾ (mg/100ml) | TG (mg/100ml) | TG (mg/g) | TG (mg/g) |
| Normal | 42.7 ± 8.5 ^{bc)} | 107.0 ± 68.3 ^b | 19.9 ± 11.9 ^{ab} | 15.2 ± 6.8 ^a |
| Diabetic -control | 97.3 ± 56.6 ^a | 334.2 ± 258.4 ^a | 34.2 ± 18.4 ^a | 15.8 ± 9.7 ^a |
| -RMJ ⁴⁾ | 52.1 ± 18.5 ^b | 128.0 ± 51.0 ^b | 6.4 ± 0.3 ^b | 13.9 ± 5.4 ^a |
| -OMJ | 70.0 ± 21.0 ^{ab} | 125.0 ± 116.7 ^b | 11.5 ± 5.8 ^b | 13.7 ± 7.2 ^a |
| -SMJ | 63.7 ± 23.3 ^b | 178.9 ± 89.0 ^b | 9.9 ± 4.5 ^b | 13.4 ± 5.6 ^a |
| -RWJ | 53.5 ± 25.1 ^b | 124.0 ± 84.1 ^b | 8.6 ± 4.6 ^b | 10.8 ± 7.7 ^a |

¹⁾Values are mean ± SEM, n=8

²⁾FFA: free fatty acid; TG: triglyceride

³⁾Within a given column, those values with different superscripts are significantly different at $p < 0.05$

⁴⁾RMJ: Raw, milled job's tears. OMJ: Roasted, milled job's tears, SMJ: Steamed, milled job's tears, RWJ: Raw, whole grain job's tears

diabetic control group, plasma free fatty acid level was significantly lower in RMJ, SMJ and RWJ, tended to be lower in OMJ. Especially, the plasma free fatty acid levels of RMJ and RWJ is close to that of normal group. The plasma triglyceride levels of all job's tears group were 50~70% lower than that of diabetic control group and close to that of normal group. The liver triglyceride levels of all job's tears group were 70~80% lower than that of diabetic control group and were even lower than that of normal group. For muscle triglyceride, no differences were seen among all groups.

DISCUSSION

Atherosclerosis is the most common complication of diabetes. Diabetic men have a two to threefold higher risk of coronary heart disease, stroke, and peripheral vascular disease, whereas diabetic women have three to fivefold higher than matched nondiabetic individuals(17). Lipoprotein abnormalities play a major role in atherosclerosis. Plasma LDL abnormalities, decreased HDL and increased triglycerides contribute to accelerated atherosclerosis in diabetes. Although the long-term effects of specific diabetic diets are not known, one should use diets that have the greatest potential for sustaining life with a minimum complications. Specifically, maintaining desirable plasma lipid levels is the key to reduce the development of these complications.

The findings that plasma total cholesterol, triglyceride and arteriosclerotic index of OMJ and SMJ were improved were consistent with the previous reports which were in normal rats(18-20). Although the fiber in the diet appears to be the one of the components responsible for lowering blood lipids, because all animals consumed the similar nutritional composition including fiber, the result of this study is from the biological activity of job's tears diet itself rather than the difference of nutritional composition between job's tears and control diet.

Because the largest reduction of total cholesterol was seen in SMJ and OMJ with the higher initial blood cholesterol levels during the experimental period, and total cholesterol levels in plasma of SMJ and OMJ were significantly lower than those of diabetic control group at the 3rd week of experimental diet, it can be suggested that the steaming and roasting are the good way of cooking for controlling plasma cholesterol profile. Several mechanisms have been suggested for lowering cholesterol such as reduced fat absorption, increased catabolism of LDL-

cholesterol and excretion of bile acids or neutral sterol(8, 21, 22). However, the lowering tendency of blood total cholesterol showed in SMJ and OMJ might be the result of the delayed digestion rates due to the viscosity because the viscosity of job's tears starch was greater than that of other grain starch(23) and the water-soluble major polysaccharide from job's tears was water soluble glucans to which are attached glucosyl side chain in a similar way to amylopectin(24) and this amylolytic digestion is also considerably slower in food containing viscous fibers(25,26).

Further support for a potential on improving lipid profile is provided by the effect of job's tears diet on triglyceride levels in plasma and liver because hypertriglyceridemia gives an higher risk of atherosclerotic cardiovascular disease on diabetic than nondiabetic individuals. Plasma and liver triglyceride levels of all job's tears groups were lower than that of diabetic control group and close to that of normal group. Compared to diabetic control group, plasma free fatty acid level was significantly lower in RMJ, SMJ and RWJ, tended to be lower in OMJ. As the results, the atherosclerotic index of OMJ and SMJ was improved compared to diabetic control group.

Thus, despite the many uncertainties regarding mode of action, these results suggest that steamed job's tears and roasted job's tears could have the potential on improving lipid profile of diabetes in clinical setting and the different cooking methods of job's tears consumed in the diet might affect the lipid profile of the diabetic rats.

ACKNOWLEDGEMENTS

This research was funded by the MAFF-SGRP(Ministry of Agriculture, Forestry, and Fisheries Special Grants Research Program), which authors feel deeply grateful.

REFERENCES

1. Otsuka, H., Hirai, Y., Nagao, T. and Yamasaki, K : Anti-inflammatory activity of benzoxazinoids from roots of Coix Lachryam-Jobi Var. Ma-Yuen. *J. Nat. Prod.*, **51**, 74(1988)
2. Tokuda, H., Matsumoto, T., Konoshima, T., Kozuka, M., Nishino, H. and Iwashima, A. : Inhibitory effects on Epstein-Barr virus activation and anti-tumor promoting activities of coix seed. *Planta Med.*, **56**, 653(1990)
3. Numata, M., Yamamoto, A., Moribayashi, A. and Yamada, H. : Antitumor components isolated from the chinese herbal medicine Coix Lachryma-Jobi. *Planta Med.*, **60**, 356 (1994)
4. Anderson, J. W. : Dietary fiber and human health. *Hort.*

- Science*, **25**, 1488(1990)
5. Schinnick, F. L., Mathews, R. and Ink, S : Serum cholesterol reduction by oats and other fiber sources. *Cereal Foods World*, **36**, 815(1991)
 6. Ripsin, C. M., Keenan, J. M., Jacobs, D. R., Elmer, P. J., Welch, R. R., VanHorn, L., Turnbull, W. H., Thye, F. W., Kestin, M., Hested, M. M., Davidson, D. M., Davidson, M. H., Dugan, L. D., Denmark-Wahnefried, W. and De-ling, S. : Oat products and lipid lowering. *JAMA*, **267**, 3317(1992)
 7. Mackay, S., Ball, M. J. : Do beans and oat bran add to the effectiveness of a low-fat diet? *Euro J. Clin. Nutr.*, **46**, 641(1992)
 8. Jenkins, D. J. A., Spadafora, P. J., Jenkins, A. L. and Rainey-Macdonald, C. G. : Fiber in the treatment of hyperlipidemia. In "CRC handbook of dietary fiber in human nutrition" Spikker, G. A.(ed.). CRC Press, Ann Arbor, MI, p. 419(1993)
 9. Woo, J. W., Lee, M. S., Lee, H. J. and Kim, H. S. : Comparative studies on the dietary fiber, amino acids and lipid components of Yullmoo and Yeomjoo, *Kor. J. Food Sci. Technol.* **21**, 269(1989)
 10. Hwang, S. H., Sung, C. J. and Kim, J. I : Analysis of dietary fiber content of common Korean foods. *J. Kor. Soc. Food Nutr.*, **24**, 396(1995)
 11. Cho, Y. and Lee, M. : The hypoglycemic effect of job's tears diet is not significant when the amount of total fiber consumption is controlled. *Kor. J. Nutr.*, **30**, 1055(1997)
 12. Thorne, M. J., Thompson, L. U. and Jenkins, D. J. A. : Factors affecting starch digestibility and the glycemic response with special reference to legumes. *Am. J. Clin. Nutr.*, **38**, 481(1983)
 13. In, S. S., Kim, M. H., Sung, C. J. and Lee, J. H. : The effect of cooking form of rice and barley on the post-prandial serum glucose and insulin responses in normal subject. *J. Kor. Soc. Food Nutr.*, **20**, 293(1991)
 14. Junod, A., Lambert, A. E. and Stauffacher, W. : Diabetogenic action of streptozotocin. Relationship of dose to metabolic response. *J. Clin. Invest.* **48**, 2129(1969)
 15. American Institute of Nutrition : Report of the American Institute of Nutrition. Ad Hoc Committee on standards for nutritional studies. *J. Nutr.*, **107**, 1340(1977)
 16. American Institute of Nutrition : Report of the Ad Hoc Committee on standards for nutritional studies. *J. Nutr.*, **110**, 1726(1980)
 17. Anderson, J. and Geil, P. : Nutritional management of diabetes mellitus. In "Modern nutrition in health and disease" Shils, M. E., Olson, J. A. and Shike, M.(eds), Lea & Feibiger, PA, p. 1266(1994)
 18. Aoki, M. and Tuzihara, N. : Effects of the hatomugi(Coix lacryma L. var ma-yuen) on the blood pressure, cholesterol absorption and serum lipids level. *Japan J. Home Economics*, **35**, 89(1984)
 19. Wuo, J., Kang, J., Park, P. and Chung, S. : Influence of dietary job's tears(Coix lacrymajobi) on lipid components in serum and liver of rats on hypercholesterolemic diet. *J. Gyeongsang Nat. Univ.*, **25**, 201(1986)
 20. Park, Y., Lee, Y. and Hiramitsu, S. : Effect of Coix on plasma cholesterol and lipid metabolism in rats. *Kor. J. Nutr.*, **21**, 88(1988)
 21. Hopewell, R., Yeater, R. and Ullrich, I. : Soluble fiber : effect on carbohydrate and lipid metabolism. *Prog. Food Nutr. Sci.*, **17**, 182(1993)
 22. Cassidy, M. M. and Calvert, R. J. : Effect of dietary fiber on intestinal absorption of lipids. In "CRC Handbook of dietary fiber in human nutrition" Spiller, G. A.(ed.), CRC Press, Ann Arbor, MI, p.162(1993)
 23. Ahn, S. W., Lee, S. W. and Choi, H. S. : A study on nutrition constituents and physical characteristics of job's tears. *J. Kor. Living Sci. Res.*, **1**, 181(1983)
 24. Yamada, H., Yanahira, S., Kiyohara, H., Cyong, J. and Otsuka, Y. : Water-soluble glucans from the seed of Coix Lacryma-Jobi Var, Ma-Yuen. *Phytochemistry*, **25**, 129 (1986)
 25. Jenkins, D. J. A., Wolever, T. M. S. and Thorne, M. J. : The relationship between glycemic response, digestibility, and factors influencing the dietary habits of diabetics. *Am. J. Clin. Nutr.*, **40**, 1175(1984)
 26. Braaten, J. T., Wood, P. J., Scott, F. W., Riedel, K. D., Poste, L. M. and Collins, M. W. : Oat gum lowers glucose and insulin after an oral glucose load. *Am. J. Clin. Nutr.*, **5**, 1425(1991)

(Received July 30, 1997)