Original Article

Mahidol University Journal of Pharmaceutical Science 2012; 39 (1), 19-24

Chemical Composition and Effects on Carbohydrate Metabolism of Chloroform Fraction of *Coix lachryma-jobi* (L) Stem Extract

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Abstract

Coix lachryma-jobi (L) seeds have been studied and used widely in many Asian countries as a mothermilk secretagogue or for nutrition, the stems are often considered as waste products, few studies on pharmaceutical benefits of *Coix lachryma-jobi* (L) stems were reported. A survey on usage of herbal medicines for the treatment of diabetes mellitus showed that *Coix lachryma-jobi* (L) stems are principal in some diabetes recipes of several Vietnamese ethnic communities. In our preliminary studies, *Coix lachryma-jobi* (L) stem ethanol extract and its chloroform fraction had hypoglycemic effect on streptozocine (STZ)-induced hyperglycemic mice. In this study, the chloroform fraction had no impact on blood glucose level and gluconeogenic enzyme activities of normoglycemic mice. On STZ-induced hyperglycemic mice, the fraction significantly reduced blood glucose level ($p<0.01$) as well as gluconeogenic enzyme activities such as G6Pase and F1,6BPase ($p<0.05$). Two compounds were isolated from the chloroform fraction and identified as β-sitosterol and stigmasterol both of which had been reported as hypoglycemic substances by various authors. This is the first time these compounds are reported as the constituents of *Coix lachrymal-jobi* (L) stem. Such results proved the hypoglycemic effect and helped to reveal partly the mechanism of *Coix lachrymal-jobi* (L) stem in treatment of diabetes mellitus.

KEYWORDS: *Coix lachryma-jobi*, Blood glucose level, Gluconeogenesis, Stem, Phytosterols

INTRODUCTION

Coix lachryma-jobi (L) seeds have been studied and used widely in many Asian countries as a mothermilk secretagogue or for nutrition, the stems are often considered as waste products, few studies on pharmaceutical benefits of *Coix lachryma-jobi* (L) stems were reported. A survey on usage of herbal medicines for the treatment of diabetes mellitus showed that *Coix lachryma-jobi* (L) stems are principal in some diabetes recipes of several Vietnamese ethnic communities[17]. In our preliminary studies, *Coix lachryma-jobi* (L) stem ethanol extract and its chloroform fraction had hypoglycemic effect on streptozocine (STZ)-induced hyperglycemic mice[13]. In this study, the effects of the chloroform fraction on blood glucose level and gluconeogenetic key enzyme activities were tested on both normoglycemic mice and STZ-induced hyperglycemic mice. Furthermore, two phytosterols were isolated from the chloroform fraction, both of which had been reported as hypoglycemic substances by various authors.

MATERIALS AND METHODS

Materials: Coix lachryma-jobi (L) stems were collected in surrounding area of Hanoi,

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Vietnam. A voucher specimen has been deposited in the Department of Pharmacognosy, Hanoi University of Pharmacy, Vietnam

Streptozocine (MP Biochemicals), Fructose1, 6 biphosphate (MP Biochemicals), Glucose 6 phosphate (Fluka biochemie Gmbh 9471), Eikonogen (Nanjing Chemlin Chemical Industry), blood glucometer Accu-check (Roche Diagnostics)

Experimental animals: male Swiss mice weighing 23-27 g obtained from the National Institute for Hygiene Epidemiology. The mice were kept in individual cages in an environmentally controlled room with a 12 h light/12 h dark cycle. The animals had free access to water and standard mice diet.

Preparation of the total extract and the chloroform fraction: The dried powdered stem of *Coix lachryma-jobi* (L.) was extracted with ethanol (80 %) then the chloroform fraction was obtained from partition of the ethanol extract.

Experimental procedures:

- Normoglycemic mice were devided into 3 groups of 10 mice each: group 1 (untreated normal mice); group 2 (normal mice treated with the total extract p.o at doses equivalent to 10 g of dried stem/kg/day for 7 days); group 3 (normal mice treated with the chloroform fraction p.o at doses equivalent to 10 g of dried stem/kg/days for 7 days). Fasting blood glucose (FBG) levels were monitored at before and after the treatment period. On the 8th day, the mice of group 1 and group 3 were sacrificed to get livers for determination of G6Pase and F1,6BPase activities.

- Hyperglycemic mice were induced by intraperitoneal injection of streptozocine at 150 mg/kg. 72h after the injection, fasting blood glucose were monitored. Those mice with FBG level exceeding 15 mmol/L were chosen as diabetic mice. The diabetic mice were divided into 3 groups of 10 mice each: group 1 (untreated diabetic mice); group 2 (diabetic mice treated with the total extract at doses equivalent to 10 g of dried stem/kg/day for 7 days); group 3 (diabetic mice treated with the chloroform fraction at doses equivalent to 10 g of dried stem/kg/days for 7 days). Blood glucose levels were monitored at before and after the treatment period. On the 8th day, the mice of group 1 and group 3 were sacrificed to get livers for determination of G6Pase and F1,6BPase activities.

Biochemical parameter assays:

- FBG level was determined by GOD method, using Accu-check blood glucometer (Roche)

- Hepatic G6Pase activity was determined by the protocol of Koida8, hepatic F1,6PBase was determined by the protocol of Gancedo3

Statistical analysis

- Data expressed as mean ± SE were analyzed using the Excel 2003. Comparisons were made by means of unpaired Student's t-test. Differences of $p < 0.05$ were considered statistically significant.

Chemical studies

- Chloroform fraction was subjected to column chromatography on silica gel (40-60 μm, Merck) with gradient elution using n-hexane : ethyl acetate. Thin layer chromatography was applied to monitor the eluates. Similar fractions were pooled together. Compound H was obtained from the n-hexane : ethyl acetate (8 : 2) fraction and was recrystallized from n-hexane.

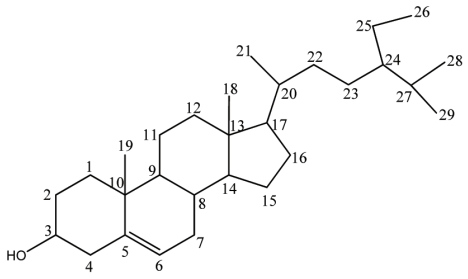
- Chemical structures of isolated compounds then were identified by NMR spectroscopies.

RESULTS AND DISCUSSION

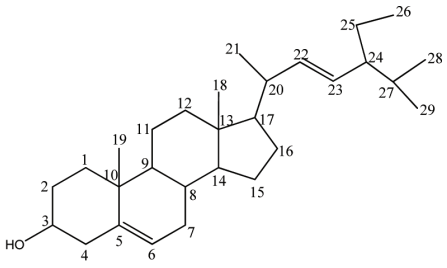
In our previous report, the ethanol total extract of *Coix lachryma-jobi* (L) stems didn't have any impact on FBG level of normoglycemic mice[13]. Similarly, in this study, after 7 days of treatment, FBG levels of both of the total extract treated group and the chloroform fraction treated group were not significantly different ($p > 0.05$) to that of the normal control group (Table 1). Furthermore, in normoglycemic mice, no changes in hepatic F1,6BPase and G6Pase were found in the treated groups (Figure 1). The results on the hepatic gluconeogenic enzyme activities were in conformity with the reported unchanged FBG levels.

Table 1. Effect of the chloroform fraction on FBG levels of normoglycemic mice

Groups	FBG level (Mean ± SE) (mmol/L)	
	Before treatment	After treatment
Normal control (1)	6.68 ± 0.75	6.72 ± 0.56
Total extract treated (2)	6.68 ± 0.75	6.72 ± 0.56
Chloroform treated (3)	6.59 ± 0.81	6.70 ± 0.66



β-sitosterol 1



Stigmasterol 2

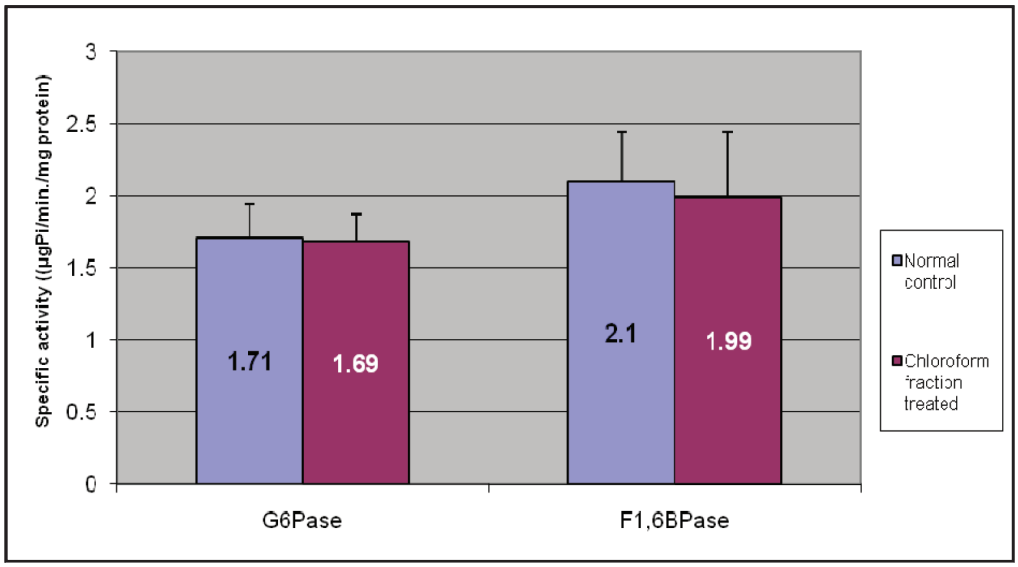


Figure 1. Effect of chloroform fraction on hepatic gluconeogenic enzyme activities in normoglycemic mice

On STZ-induced hyperglycemic mice, the chloroform fraction decreased significantly the FBG level ($p < 0.01$ vs. diabetic control). The FBG level of the fraction treated group was equivalent to that of the total extract group ($p > 0.05$) (Table 2).

Table 2. Effect of the chloroform fraction on FBG *(*): p<0.01 compare with control*

Groups	FBG level (Mean ± SE) (mmol/L)	
	Before treatment	After treatment
STZ control (1)	19.86 ± 2.28	18.82 ± 3.40
Total extract treated (2)	20.54 ± 3.09	10.56 ± 2.25*
Chloroform treated (3)	20.08 ± 3.42	10.74 ± 1.21*

A similar result on hepatic gluconeogenic enzyme activity was obtained. G6Pase and F1,6BPase are rate-limiting enzymes in the gluconeogenesis pathway, the primary production of free glucoses for the blood and thus plays an essential role in blood glucose level homeostasis[10, 15]. It's reported that in diabetic patients and in experimentally induced diabetic animals, high blood glucose levels were accompanied by significant increases of gluconeogenic enzyme activities[1]. Therefore, G6Pase and F1,6BPase have been considered as potential targets for antidiabetic agents[4]. In fact, several antidiabetic plant were proved to inhibit gluconeogenic enzymes[7, 14, 16]. In our study, the chloroform fraction lowered significantly hepatic G6Pase and F1,6PBase activities of hyperglycemic mice ($p < 0.05$) (Figure 2). This result partly helped to explain the hypoglycemic effect of the chloroform fraction in STZ injected mice.

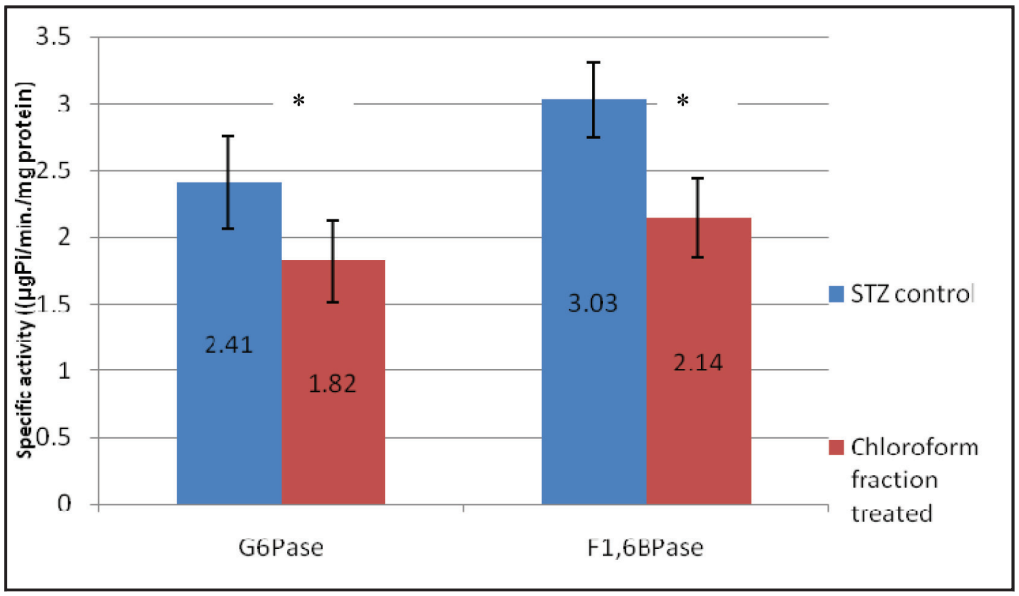


Figure 2. Effect of chloroform fraction on gluconeogenic enzyme activity in hyperglycemic mice *(*): $p < 0.05$*

As chloroform fraction was the most potent fraction and showed antidiabetic activity with equivalent level as the total extract, we therefore made attempt to isolate and identify active compound present in this fraction. Consequently, a compound named compound H (white crystalline substance) was successfully isolated. H yielded a single spot when subjected to thin layer chromatography using several solvent systems including dichloromethane : methanol (100 : 0.6), n-hexane : ethyl acetate (7 : 3), toluene :

ethyl acetate (3 : 7), with vanillin - H_2SO_4 reagent. H showed positive results in Liebermann-Burchard reaction for steroidal nucleus. The ^{1}HNMR, ^{13}CNMR spectral data and a comparison of the ^{1}HNMR and ^{13}CNMR signal with those described in the literatures[5, 12] showed the structure of H to be the mixture of β-sitosterol **1** and stigmasterol **2**. The only difference between the two compounds is the presence of C22 - C23 double bond in stigmaterol and C22 - C23 single bond in β-sitosterol. Thus, their physical properties of are so nearly identical that it's difficult to separate them[2]. With regard to antihyperglycemic activity mentioned above, previous studies revealed that stigmasterol reduced serum glucose concentrations by reducing the activity of hepatic glucose-6-phosphatase with concomitant increase of circulating insulin level[11]. β-sitosterol has been also demonstrated to reduce serum glucose concentration both in normal and hyperglycemia rats by improving the oral glucose test with an increase in glucose-induced insulin secreation[6]. Taken together, it could be speculated that phytosterols such as stigmasterol and β-sitosterol play an important role for antihyperglycemic activity of the chloroform fraction from *Coix lachryma-jobi* (L) stems.

CONCLUSION

Our results proved the hypoglycemic effect and helped to reveal partly the mechanism of *Coix lachrymal-jobi* (L) stem in treatment of diabetes mellitus.

ACKNOWLEDGEMENTS

The authors wish to thank The Dept. of Science and Technology of Haiduong province for funding our reseasch.

Table 3. ^{1}H and ^{13}C NMR data of H (measured in $CDCl_3$ (500 MHz)

	β-sitosterol		Stigmasterol			β-sitosterol		Stigmasterol	
No	**δH (ppm)**	**δC (ppm)**	**δH (ppm)**	**δC (ppm)**	**No**	**δH (ppm)**	**δC (ppm)**	**δH (ppm)**	**δC (ppm)**
1		37,3		37,3	**16**		28,3		28,9
2		31,7		31,7	**17**		56,0		56,9
3	3,54 (m)	71,8	3,54 (m)	71,8	**18**	0,70 (s)	11,9	0,70(s)	12,3
4		42,2		42,2	**19**	1,05 (s)	19,4	1,05 (s)	19,4
5		140,8		140,8	**20**		36,2		40,5
6	5,38 (d, *J* = 4 Hz)	121,7	5,38 (d, *J* = 4 Hz)	121,7	**21**	0,93 (d, *J* = 6,4 Hz)	18,8	0,93 (d, *J* = 6,4 Hz)	21,2
7		31,5		31,5	**22**		33,9	5,18 (dd , *J* = 15 Hz ; 6,5 Hz)	138,3
8		31,9		31,9	**23**		26,1	5,03 (dd , *J* = 15 Hz ; 6,5 Hz)	129,3
9		50,1		50,1	**24**		45,8		51,2
10		36,5		36,5	**25**		29,1		33,7
11		21,1		21,2	**26**		19,4		21,1
12		39,7		39,8	**27**		19,0		21,2
13		42,3		42,3	**28**		23,1		26,1
14		56,8		56,9	**29**		12,1		12,1
15		24,3		24,4					

REFERENCES

1. Clore JN., Stillman J., Sugerman H. Glucose-6-phosphatase flux *in vitro* is increased in type 2 diabetes, *Diabetes* 2000:49(6): 969-74.
2. Fieser L.F., Fieser M. Organic Chemistry 3rd Ed. Wiley New York: 250-353.
3. Gancedo JM, Gancedo C. Fructose 1, 6-bisphosphatase, phosphofructo-kinase and glucose 6-phosphate dehydrogenase from fermenting and non-fermenting yeasts. *Arch Mikrobiol* 1971:76: 132-8.
4. Henga , S. Gryncela RK., Kantrowitz ER. A library of novel allosteric inhibitors against fructose 1,6-bisphosphatase, *Bioorg Med Chem.* 2009:17(11): 3916-22.
5. Huang L., Cao Y., Xu H., Chen G. Separation and purification of ergosterol and stigmasterol in Anoectochilus roxburghii (Wall) Lindl by high-speed counter-current chromatography. *Separ Sci* 2011: 34: 385-92.
6. Ivorra M.D., D'Ocon M.P., Paya M., Villar A. Antihyperglycemic and insulin-releasing effects of beta-sitosterol 3-beta-D-glucoside and its aglycone, beta-sitosterol"; *Arch Intern Pharmacodyn Therapie* 1988: 296: 224-31.
7. Kannappan S, Jayaraman T, Rajasekar P, Ravichandran M K, Anuradha C V. Cinnamon bark extract improves glucose metabolism and lipid profile in the fructose-fed rat. *Singapore Med* J 2006 : 47(10):858 - 63.
8. Koida H, Oda T. Pathological occurrence of glucose-6-phosphatase in liver disease. *Clin Chem Acta* 1959: 4:554-61.
9. Lowry, O. H., N. J. Rosebrough, A.L. Farr and R. J. Randall. Protein measurement with the Folin-Phenol reagents. *J Biol Chem* 1951:193: 265-75.
10. Mogale MA., Lebelo SL., Shai LJ. Aloe arborescens aqueous gel extract alters the activities of key hepatic enzymes and blood concentration of triglycerides, glucose and insulin in alloxan-induced diabetic rats. *African Biotechn* 2010: 10(20): 4242-48.
11. Panda S., Jafri M., Kar A., Meheta B.K. Thyroid inhibitory, antiperoxydative and hypoglycemic effects of stigmasterol isolated from Butea monosperma, *Fitoterapia* 2009:80(2): 123-26.
12. Pateh U. U, Haruna A. K., Garba M., Iliya I., Sule I. M., Abubakar M. S., Ambi A. A. Isolation of stigmasterol, β-sitosterol and 2-hydroxyhexadecanoic acid methyl ester from the rhizomes of Stylochiton lancifolius Pyer and Kotchy (Araceae). *Nigerian Pharm Sci* 2009:8(1):19-25.
13. Phung Thanh Huong, Nguyen Thi Dong, Dang Thai Trung. Hypoglycemic activity of Coix lachrymal-jobi in mice. Oral presentation at the Youth Conference of Science and Technology XV: 160-65.
14. Phung Thanh Huong, Nguyen Xuan Thang, Do Ngoc Lien. Effects of banaba leaf and n-hexan fractions on carbohydrate metabolism in mice livers. Oral presentation in Indochina Conference on Pharmaceutical sciences 2009:258-61.
15. Reed MJ, Mezaros K, Entes LJ, Claypool MD, Pinkett JG, Brignetti D, *et al*. Effect of masoprocol on carbohydrate and lipid metabolism in a rat model of Type II diabetes. *Diabetologia* 1999:42: 102-6.
16. Soon YY., Tan BKH. Evaluation of the Hypoglycemic and Anti-Oxidant Activities of Morinda officinalisin Streptozotocin-induced Diabetic Rats. *Singapore Med* J 2002:43(2): 77-85.
17. Tran VO, Phung TH. Antidiabetic plants in Vietnam, Report on researches under the fund of Ministry of Health 2010.