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THE OPTIMAL EXTRACTION PARAMETERS AND ANTI-DIABETIC ACTIVITY OF
FLAVONOIDS FROM IPOMOEA BATATAS LEAF

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Abstract

Extraction parameters of flavonoids from *Ipomoea batatas* leaf (FIBL) and anti-diabetic activity of FIBL on alloxan induced diabetic mice were studied. The optimal extraction parameters of FIBL were obtained by single factor test and orthogonal test, as follows: ethanol concentration 60 %, ratio of solvent to raw material 30 , extraction temperature 75 °C and extraction time 1.5 h , while extraction yield of FIBL was 5.94 %. FIBL treatment (50, 100, and 150 mg/ kg body weight) for 28 days resulted in a significant decrease in the concentration of fasting blood glucose (FBG), total cholesterol (TC) and triglyceride (TG) in diabetes mellitus mice. Furthermore, FIBL significantly increased body weight (bw) and serum high-density lipoprotein cholesterol (HDL-c) level. The data demonstrated FIBL at the dose of 100 mg/kg bw exhibited the optimal effect. The above results suggest that FIBL can control blood glucose and modulate the metabolism of blood lipid in diabetes mellitus mice.

Key words: flavonoids from *Ipomoea batatas* leaf; extraction; anti-diabetic.

Introduction

Diabetes mellitus is a metabolic disorder of the endocrine system. The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose (Kumar et al., 2008). Diabetes is becoming the third 'killer' of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality (Li et al., 2004). Approximately 4% of the population worldwide is affected and expected to increase by 5.4% in 2025 (Kim et al., 2006). These facts show that proposing an immediate strategy for diabetes prevention and treatment is a global subject. For a long time, diabetics have been treated with several medicinal plants or their extracts based on the folklore medicine (Akhtar and Ali, 1984). Synthetic hypoglycemic agents can produce serious side effects and they are too expensive. Therefore, the search

for more effective and safer hypoglycemic agents has continued to be an important area of active research.

Ipomoea batatas leaf possess accelerating metabolism, preventing arteriosclerosis, protecting eyesight, hypoglycemic and antimutation activities (Han, 2000). Flavonoids is considered to be one of main active components of *Ipomoea batatas* leaf. (Harborne and Williams, 2000). The health benefits of flavonoids are well known and are displayed as a remarkable range of biochemical and pharmacological properties (Middleton *et al.*, 2000). Accumulating evidence shows that flavonoids have antioxidant, hypolipidaemia, soften the blood vessel, hypoglycaemia properties (Hollman *et al.*, 1999), and reduced risk for developing various cancers (Carroll *et al.*, 1998; Sharma *et al.*, 2008). But at present there is no study on the effect of flavonoids extracted from *Ipomoea batatas* leaf on diabetes mellitus. The purpose of this study was to investigate optimal extraction parameters of FIBL and Anti-diabetic activity of FIBL on alloxan-induced diabetic mice for the use of this plant in the treatment of diabetes.

Materials and methods

Plant material

Leaves of *Ipomoea batatas* were collected from Hebei province district in the month of October and the material was identified by Mr Wang GuangYao, a botanist of Jilin Agriculture Science and Technology College. A voucher specimen has been deposited in herbarium of Jilin Agriculture Science and Technology College. Fresh, intact, leaves were picked to shade dried as experimental material.

Drugs and reagents

Rutin standard was obtained from Chengdu mansite pharmaceutical Co. (Chengdu, China). Alloxan was obtained from Sigma Co. (USA). Glucose Analyzer and strips were purchased from Roche Diagnostic Co. (USA). Reagents for TC, TG and HDL-c were obtained from Beijing Chengxinde Biochemistry Reagent Co. (Beijing, China). All other reagents were of analytical grade.

Extraction of FIBL

The shade dried *Ipomoea batatas* leaf was crushed in an electrical grinder and then powdered. Out of this powder, 10g was subjected to hot continuous extraction in Erlenmeyer flask with ethanol (ethanol concentration ranged from 40 to 90 %, ratio of solvent to raw material ranged from 10 to 40), while the temperature of the water bath ranged from 30 to 90 °C and was kept steady (within ± 1.0 °C). After a period of time (ranged from 0.5 to 3 hrs), a deep brown extract was obtained which was filtrated by using the filter. The extract was evaporated by using a rotary evaporator (RE52AA, Yalong Biochemical Instrument Co., Shanghai, China) under reduced pressure at 40 °C to get the flavonoids. It was stored at (0-4) °C until used. The contents of flavonoids were determined by the NaNO_2 - $\text{Al}(\text{NO}_3)_3$ - NaOH colorimetric assay and by reference to Rutin, and wavelength in spectrophotometer (V-5100, Beijing Chenxiyongchuang Science and Technology Co., Beijing, China) was set at 510 nm (Xu, 2007; Wang *et al.*, 2004).

Experimental animals

Male mice of original Kun-ming strain, weighing 18-22 g, were used for the study, housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25 ± 30 °C, 35-60 % humidity), the animals were fed with standard diet and water *ad libitum*. The approval of this experiment was obtained from the Institutional Animal Ethics Committee of Yanshan University (Qinhuangdao, China). After 1 week of acclimation, the fasted mice were induced with a single injection of 4 % alloxan prepared freshly at a dose of 200 mg/kg bw (Yang *et al.*, 2006). Diabetes was confirmed by the determination of tail vein blood glucose levels on the third day after administration of alloxan. Mice having blood glucose levels greater than 11.1 mmol/L were considered diabetic and were used for the study (Yang *et al.*, 2006; Dong *et al.*, 2006)..

Anti-diabetic activity of FIBL

Sixty male Mice were randomly divided into six equal groups as follows:

- i) Normal control group (NC): normal control mice administered water daily for 28 days;
- ii) Diabetic control group (DC): diabetic control mice administered water daily for 28 days;
- iii) Diabetic + FIBL (150 mg/kg) group (DHF): diabetic mice administered flavone extracted from *Ipomoea batatas* leaf (150mg/kg) daily for 28 days.;
- iv) Diabetic + FIBL (100 mg/kg) group (DMF): diabetic mice administered flavone extracted from *Ipomoea batatas* leaf (100 mg/kg) daily for 28 days.;
- v) Diabetic + FIBL(50 mg/kg) group (DLF): diabetic mice administered flavone extracted from *Ipomoea batatas* leaf (50 mg/kg) daily for 28 days.;
- VI) Diabetic + glibenclamide (0.25 mg/kg) group (DG): diabetic mice administered reference drug glibenclamide (0.25 mg/kg) daily for 28 days.

During FIBL and glibenclamide supplement for 28 days, fasting blood glucose level was measured once every week. Blood was collected from tip of the tail vein and fasting blood glucose level was measured by using a glucose analyzer. At the same time, the body weight of each mouse was measured with a weighing balance (MP2000, Shanghai instrument No.1 factory, Shanghai, China). On 28th day of experiment, the mice were sacrificed by decapitation under light ether anesthesia and blood was collected from dorsal aorta and serum was separated by centrifugation for 5 min (VORTEX21K, Xiangyi centrifuge machines Co., Changsha, China). TC ,TG and HDL-c were determined by enzyme assay methods. All results were expressed as mean \pm SD and analyzed by SPSS for Windows (version 13.0, SPSS Inc, Chicago, USA). The Duncan test and one way analysis of variance were used for comparisons (Achyut et al., 2007). The values were considered significant when $P < 0.05$.

Acute toxicity studies

Healthy mice (18–22 g) of either sex, starved overnight were divided into three groups ($n = 6$) and were orally fed with FIBL in increasing dose levels of 300, 900, 1500 mg/kg bw. The animals were observed continuously for 2 hrs under the following profiles (Shirwaikar et al., 2006):

- i) Behavioral profile. Alertness, restlessness, irritability, and fearfulness.;
 - ii) Neurological profile. Spontaneous activity, reactivity, touch response, pain response and gait;
 - iii) Autonomic profile. Defecation and urination.
- After a period of 24 and 72 hrs they were observed for any lethality or death.

Results and Discussion

Different extraction conditions, such as ethanol concentration, ratio of solvent to raw material, extraction temperature and extraction time, have different effects on extraction yield of flavonoids (Zhang et al., 2007). Ethanol concentration is an important factor affecting extraction yield of flavonoids (Lan et al., 2007), which was set at 40 %, 50 %, 60 %, 70 %, 80 % and 90 %, respectively. With the increase of ethanol concentration from 40 % to 60 % in the extraction system, the extraction yield quickly increased from 3.41% to 5.17% (Figure 1). However, with the increase of the ethanol concentration from 60% to 90% in the extraction system, extraction yield quickly decreased from 5.17 % to 2.94 %. This maybe because the extraction efficiency of water-soluble flavonoids and alcohol-soluble flavonoids is best when extracted with 60% ethanol. Therefore, the suitable ethanol concentration for higher yield of the FIBL was considered to be 60%

Ratio of solvent to raw material was another factor affecting extraction yield of flavonoids (Zhang et al., 2007). It was set at 10, 15, 20, 25, 30, 35 and 40, respectively. With the increase of ratio of solvent to raw material from 10 to 30 in the extraction system, extraction yield quickly increased from 4.21 % to 5.31 %. It can be seen from Figure 2 that extraction yield of flavonoids in the extraction system gives higher value when ratio

of solvent to raw material was 30. In addition, extraction yield of flavonoids decreased from 5.31 % to 3.69 % when ratio of solvent to raw material was from 30 to 40 in the extraction system. Therefore, the suitable ratio of solvent to raw material for higher total yield of the FIBL was considered to be 30.

As for the extraction temperature, the higher the temperature, the higher the extraction yield of flavonoids. As shown in Figure 3, extraction yield increased from 3.98 % to 5.65 % with the increasing temperature. However, on the other hand, a relatively high extraction temperature (at 90 °C) was detrimental to the extraction yield. Only a bit of the extraction yield was increased as the temperature was higher than 70 °C because of destruction of enzyme activity at high temperature in this reaction system (Zhao et al., 2007). Therefore, the suitable temperature for higher yield of the FIBL was considered to be 70 °C.

Extraction time is another factor that would influence the extraction efficiency and selectivity of the fluid (Zhu 2006). With the increase of the extraction time from 1 to 2 hrs in the extraction system, the extraction yield quickly increased from 3.27 % to 5.61 % (Figure 4). When extraction time continued to lengthen, the extraction yield increased a little. Because longer extraction time could delay and lengthen production cycle, 2 hrs of extraction time was adopted in the present work.

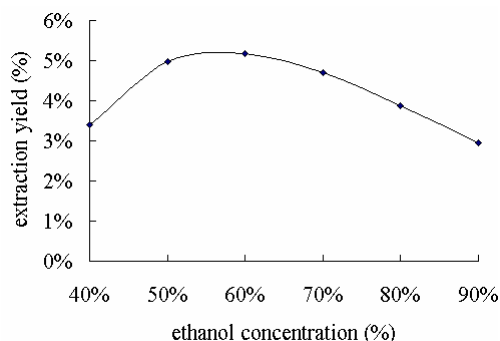


Figure 1: Effect of ethanol concentration on extraction yield of FIBL

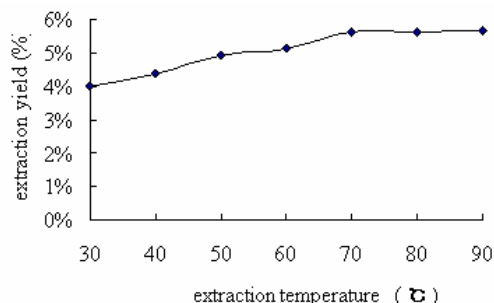


Figure 3: Effect of extraction temperature on extraction yield of FIBL

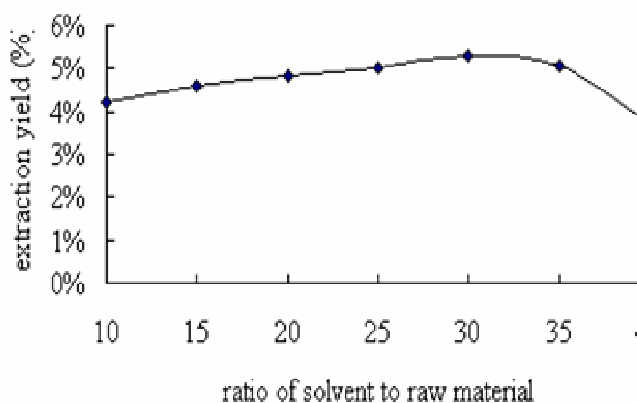


Figure 2. Effect of ratio of solvent to raw material on extraction yield of FIBL

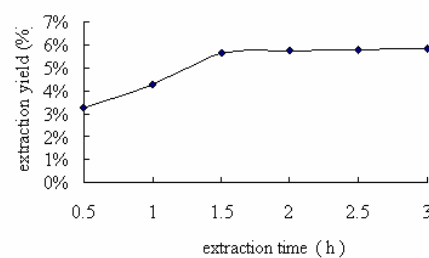


Figure 4: Effect of extraction time on extraction yield of FIBL

Table 1 Factors and levels of orthogonal test

No	Factor A (ethanol concentration) (%)	B (ratio of solvent to raw material)	C (extraction temperature) (°C)	D (extraction time) (h)
1	55	25	65	1.5
2	60	30	70	2
3	65	35	75	2.5

Table:2 $L_9(3)^4$ orthogonal test result

No.	A (ethanol concentration) (%)	B (Ratio of solvent to raw material)	C (extraction temperature) (°C)	D Extraction time (h)	extraction yield (%)
1	1	1	1	1	5.08
2	1	2	2	2	5.39
3	1	3	3	3	5.24
4	2	1	2	3	5.21
5	2	2	3	1	5.94
6	2	3	1	2	5.27
7	3	1	3	2	5.06
8	3	2	1	3	5.24
9	3	3	2	1	5.01
K_1	5.237	5.117	5.197	5.343	
K_2	5.473	5.523	5.203	5.240	
K_3	5.103	5.173	5.413	5.230	
R	0.370	0.406	0.216	0.113	

Table 3: Effect of FIBL on body weight (g) in mice

Group	Animal number	Days after dosing				
		0d	7d	14d	21d	28d
NC	10	19.01±0.66	23.33±0.58	25.50±0.49	27.66±0.39	30.21±0.59
DC	10	19.04±1.03	20.53±1.18 *	22.14±1.08 *	23.11±0.91 *	23.74±0.83 *
DHF	10	19.37±0.74	21.30±1.26 *	23.17±1.25 *	24.95±1.23 #	25.82±1.14 *
DMF	10	19.10±1.07	21.42±1.75 *	23.87±1.34 *	25.81±1.79 #	27.37±1.55 *
DLF	10	19.04±1.04	20.86±1.55 *	22.57±1.66 *	24.02±1.49 #	25.21±1.34 *
DG	10	19.10±0.99	21.77±1.38 #	25.33±0.69 #	27.32±0.83 #	29.30±1.22 #

n=10; (mean±S.D., g); * $P < 0.05$ as compared with normal control group. # $P < 0.05$ as compared with diabetic control Group

Table 4: Effect of FIBL on blood glucose level (mmol/L) in mice

Groups	Animal number	Days after dosing			
		0 d	7 d	14 d	28 d
NC	10	5.92±1.79	6.23±1.75 *	5.91±1.57 *	6.43±1.72 *
DC	10	22.19±3.45 *	22.51±2.11 *	22.60±3.39 *	22.41±2.71 *
DHF	10	22.15±3.45 *	19.27±2.22 * #	16.75±1.95 #	14.56±2.37 * #
DMF	10	22.70±3.40 *	17.20±2.34 * #	13.83±2.78 #	9.72±2.22 * #
DLF	10	22.29±3.37 *	19.34±2.66 * #	16.99±2.45 #	14.34±2.53 * #
DG	10	22.72±3.70 *	11.52±1.71 * #	9.91±2.15 * #	7.08±1.08 #

n=10; (mean±S.D., g); * $P < 0.05$ as compared with normal control group.; # $P < 0.05$ as compared with diabetic control Group

Table 5: Effect of FIBL on blood lipids (mmol/L) in mice

Groups	Animal number	TC	TG	HDL-c
NC	10	2.61±0.17	1.64±0.25	1.65±0.16
DC	10	3.43±0.56 *	3.33±0.16 *	0.76±0.21 *
DHF	10	3.15±0.16 * #	2.45±0.53 * #	0.97±0.13 * #
DMF	10	2.84±0.21 #	1.77±0.31 #	1.21±0.15 * #
DLF	10	3.21±0.12 *	2.78±0.11 * #	0.82±0.12 *
DG	10	2.75±0.57 #	1.79±0.22 #	1.44±0.28 * #

n=10; (mean±S.D., g); * $P < 0.05$ as compared with normal control group.; # $P < 0.05$ as compared with diabetic control Group

Orthogonal test with four factors and three levels to analyze the optimal process parameters of extraction yield. And the $L_9 (3)^4$ table was designed to detect the most suitable extraction conditions of four factors (ethanol concentration, ratio of solvent to raw material, extraction temperature and extraction time) for extraction yield (Table 1). According to the value of range R in Table 2, ratio of solvent to raw material (factor B) exerted the most significant effect on extraction yield, and the order of importance that influenced extraction yield was found to be ratio of solvent to raw material (B) > ethanol concentration (A) > extraction temperature (C) > extraction time (D). The optimal combination parameters of the processing technology were $A_2B_2C_3D_1$,

namely, ethanol concentration (60 %), ratio of solvent to raw material (30), extraction temperature (75 °C) and extraction time (1.5 h), while extraction yield of FIBL was 5.94 %.

Acute toxicity studies revealed no obvious symptom of toxicity or any significant changes in general behaviour in mice exposed to FIBL. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

The alloxan-induced diabetic mice exhibited loss of body weight. Before embarking on the experiment, all the groups had no significant difference in body weight ($P > 0.05$). A significant ($P < 0.05$) decrease in body weight was detected in diabetic control group as compared to the normal control group from 7 days after alloxan injection. However, the body weights in the DMF mice were significantly ($P < 0.05$) and dose-dependently increased as compared to those of the diabetic control from 14 days after administration. In the DG group, a significant ($P < 0.05$) increase in body weight as compared to the diabetic controls was also detected from 7 days after administration. The results are shown in Table 3.

Diabetes mellitus is a serious chronic disease, and effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both type 1 and type 2 diabetic patients (Abraira et al., 1995; DeFronzo, 1999). In the diabetic control group, a significant ($P < 0.05$) increase in blood glucose levels was detected as compared to the normal control group, but these abnormal increases in blood glucose levels significantly ($P < 0.05$) and dose-dependently decreased in the FIBL-administered groups as compared to the diabetic control group at 7 days after administration. In the DG group, a significant ($P < 0.05$) decrease in blood glucose levels as compared to the diabetic control group was also detected from 7 days after administration. The dosage of 100mg/kg is more effective than that of 50 mg/kg and 150 mg/kg. The results are shown in Table 4.

Diabetes is also associated with hyperlipidemia (Maiti et al., 2005). The present results showed that the TC and TG levels were significantly elevated in the diabetic control group as compared to the normal control group ($P < 0.05$), and serum HDL-c level, a friendly lipoprotein, was decreased in diabetic control group as compared to the normal control group ($P < 0.05$). After supplementation with the FIBL and glibenclamide, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum TG and TC levels and by increased HDL-c concentration in diabetic mice. The response was better in the DMF group compared with the DLF group and the DHF group which is comparable to that of the DG group. The results are shown in Table 5.

Our research has indicated that FIBL possesses antidiabetic activities and the dose of 100 mg/kg body weight represents the optimal level for effecting a positive diabetic response in mice. Therefore, FIBL should be considered as a candidate for future studies on diabetes. Further studies are in progress to elucidate the molecular and cellular mechanism of FIBL.

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