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Effect of Sweet potato (*Ipomoea batatas* (L.) Lam) leaf extract on hypoglycaemia, blood insulin secretion, and key carbohydrate metabolic enzymes in expermentally obese and STZ-induced diabetic mice

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Abstract. Hypolipidemic, hypoglycaemic effects of the ethyl acetate extract fraction from leaves of sweet potato (*Ipomoea batatas* (L) Lam.) (Convonvulaceae) in obese and streptozotocin (STZ) induced type 2 diabetic mice were demonstrated. When obese-diabetic mice was administrated orally daily by ethyl acetate fraction of 1000mg lyophilized powder/kg for 21 days, we showed that maximum hypoglycaemic(36.77%) and hypolipidemic effects, such as TC(35.18%), TG(29.17%), and LDLc(51.97%) were proven in treated mice compared to the control (untreated mice). The hypoglycaemic effects of ethyl acetate extract fraction from leaves of sweet potato so that it accelerated hexokinase activity, stimulated insulin secretion and inhibited gluconeogenesis enzymatic activity (glucose-6-phosphatase).

Keywords: Ipomoea batatas leaf, obese mice, type 2 Diabetes mellitus, hypolipidemic and hypoglycaemic effect.

1. Introduction

Obesity and diabetic mellitus (DM) were the diseases among the most common metabolic disorder in developed and developing countries. The disease is increasing rapidly in most parts of the world. In 2008, the World Health Organization reported that there are approximately 1.7 billion overweight and obese persons and over 200 million persons suffered from diabetes mellitus (DM), and this number will increase in future, about 330million by 2025 [1]. Abnormalities in blood lipid profile are the cause by origin and simultaneously the most common complication of DM. Besides drugs classically used for the treatment of diabetes (Insulin, sulphonylureas, biguanides and thiazolidinediones), several species of plants having a hypoglycemic and hypolipidemic activity have been described in

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the traditional remedies and scientific reports [1,2].

Sweet potato (*Ipomoea batatas* (L.) Lam) was grown popularly in many countries and was one of important crops in the world. Its leaves, the by-products, possess activities of accelerating metabolism, preventing arteriosletosis, protecting eyesight, hypoglycaemia and anti-oxidant [2,3]. Flavonoid is considered to be one of main bioactive components of *Ipomoea batatas* leaf [3]. In the world, there some studies on bioactive components and the effects of natural compound extracted from *Ipomoea batatas* leaf on diabetes mellitus. In Vietnam, sweet potato was important crops and was grown popularly every where in the country but up to now, there is no study on anti-obesity and hypoglycemic effects on the basis of the key enzymes activity of carbohydrate metabolism. The purpose of this study is to investigate hypolipidemic and hypoglycaemic effects of extract fractions from *Ipomoea batatas* leaves.

2. Materials and methods

2.1. Plant material and preparation of sweet potato leaf extract

Fresh sweet potatoes (*Ipomoea batatas* (L) Lam) leaves were collected after the classification made by Department of Botany, Vietnam National University, Hanoi, collected plant materials were washed thoroughly with water, dried at 50^0C and grinded into powder.

Powdered samples were extracted repeatedly three times with 10 volumes of 90% ethanol by continuous stirring. The extract was filtered and lyophilized to obtain ethanol extract concentrate (EtOH). This concentrate was dissolved in distilled water (1:2, w/v) and was partitioned in turn via n-hexan, chloroform, ethyl acetate, n-butanol solvents. These extract fractions were lyophilized to obtain n-hexan(Hex), Chloroform (Chlf), ethylacetate (EtOAc), concentrates. All the concentrates was stored at -20^0C until use.

2.2. Animals

Male Swiss mice obtained from the National Institute of Hygiene and Epidemiology (NIHE), weighing 14-16g, was used for the experiments. The animals was housed at 25 ± 4^0C with 12h light and dark cycle . All the mice were divided into two lots, one fed with normal diet (ND from NIHE), other fed with high fat diet (HFD) [4,5] and water *ad libitum*, for 6 weeks.

2.3. Development of HFD-fed and STZ-induced type 2 diabetic mice

After 6 weeks of dietary manipulation, a subset of the mice from each dietary group was injected intraperitoneally (i.p) STZ with dose of 120mg kg^{-1} (freshly prepared in 0,1M Citrate buffer, pH 4.5). Control lots of (ND and HFD mice) were injected with the citrate buffer alone. 72 hours after STZ injection, the blood fasting glucose of all the mice was monitored. Only STZ-treated mice with blood fasting glucose greater than 324mg/dl (18mmol/l) were considered to be diabetic and used in this study [4,6].

2.4. Treatment of obese and diabetic mice by extract fractions from sweet potato leaves

The obese and diabetic mice were treated orally daily for 21 days with 1000mg/kg of lyophilized extract fractions from sweet potato leaves. The controls were ND and obese diabetic untreated mice.

2.5. Blood and liver collection and biochemical analysis

The blood of mice fasted for 12h was collected from retro-orbital plexus using capillary tubes in to eppendorf tubes containing

heparin. The plasma was separated by centrifugation for 5 min. at 1200 rpm/min. Mice livers was quickly removed and washed with cold 0.9% saline and stored at -20^{0}C until use [7]. Blood fasting glucose was determined by automatic glucose analyzer (One touch Ultra, USA). Plasma insulin (PI) concentration was determined by enzyme immune assay kit (Mercodia, Sweden). Total cholesterol (TC), triglycerides (TG), LDL –cholesterol (LDLc), HDL-cholesterol (HDL-c) was mearsured by automatic analyzer OLYMPUS AU-400 (Japan) using a commercial diagnostic kits. Hepatic hexokinase and glucose-6-phosphatase activity were determined by method of Brandstrup [7,8].

Statistical analysis.

All values are expressed as mean ± S.E.M. Statistical significance of the difference between groups was determined by analysis of variance (ANOVA) followed by Ducan's test. A value of p< 0.5 was considered to be statistically significant.

3. Results and discussion

3.1. Body weight, biochemical parameters of ND and HFD fed mice

Table 1 indicated that the body weight, blood lipid parameters, such as TC, TG, LDTc, and plasma insulin concentration (pmol/l) in HFD fed mice increased clearly after 6 weeks of dietary manipulation as compared to the control (ND mice). While, HDLc in HFD mice decreased 34.63% in comparision with the control (ND mice). Moreover, blood glucose and insulin concentration unusually increase in obese mice in comparison with the control (ND mice). Namely, blood glucose level increased by 48.11% and plasma insulin icreased by 122.36% in HFD mice. The results showed that the model of experimental obese mice was established successfully (table 1).

Table 1. Effect of high fat diet on body weight, plasma insulin and lipid parameters

	ND	HFD	Change,%
Starting point BW	14.31 ± 1.28	14.63 ± 1.52*	↑2.23
Final BW	32.86 ± 3.92	53.21 ± 4.62*	↑61.93
TC (mg/dL)	105.14 ± 8.52	196.03 ± 10.36*	↑86.45
TG (mg/dL)	92.17 ± 4.69	183.59 ±7.36*	↑99.19
LDL-c (mg/dL)	54.34 ± 3.83	138.12 ± 6.21*	↑154.18
PI(pmol/l)	218.16 ± 13.63	485.12 ± 17.71	↑122.37
HDL-c(mg/dL)	32.37±3.51	21.16±2.53*	↓34.63
Glucose (mmol/l)	5.47±0.35	9.42±0.37	↑48.11

Values are means ± S.E.M; n=10 in each group; *: indicates significant difference (p<0,05)ND: normal diet; HFD: high fat diet; TC: total cholesterol, TG: triglyceride, PI: plasma insulin, BW: body weight, HDLc: high density lipoprotein associated cholesterol, LDLc : low density lipoprotein asociated cholesterol.

3.2. Effect of STZ injection on ND-fed and HFD fed mice after 72h

STZ is the toxin from Actinomycetes (*Streptomyces chromogen*). It was used to induce experimentally diabetic models of animals [4,5,7]. The injection of single dose of STZ (120mg kg^{-1}) into the HFD mice increases clearly blood glucose, TC, TG, LDLc and PI levels in HFD fed mice compare to the other mice (Table2).

Table 2. Effect of STZ (120mg/kg) on ND- and HFD –fed mice

	ND	ND +STZ	HFD	HFD +STZ
Body weight	33.17 ±3.24	36.42 ±3.58	51.94 ±3.17*	46.71 ±3.5**
Glucose(mmol/l)	6.36 ±0.22	6.69 ±0.12	9.42 ±0.45	23.24 ±0.47**
TC (mg/dL)	101.03 ±3.43	110.11 ±5.60	202.14 ±5.47*	267.43 ±7.75**
TG (mg/dL)	90.54 ±5.76	87.02 ±1.91	140.27 ±2.66*	651.73 ±2.08**
LDL-c (mg/dL)	54.34 ±3.83	62.20 ±2.48	224.64 ±7.14*	125.24 ±7.14**
PI(pmol/l)	232.32 ±2.00	217.63 ±3.42	467.50 ±32.43	241.72 ±26.31**
HDL-c (mg/dL)	33.07 ±4.51	30.50 ±3.21	19.77 ±2.49*	11.84 ±1.94**

Values are mean SEM, *: $p < 0.05$ vs. ND group; **: $p < 0.05$ vs. HFD group

Especially, there are significant changes of these parameters in obese mice treated with STZ (120mg/kg), such as glucose and TG levels increase approximately 2.46 and 4.64 times respectively in comparison with HFD fed mice untreated with STZ. It is clear that the diabetic STZ induced HFD fed mice were expressed diabetic disease and a insulin resistance. However, plasma insulin in HFD-fed mice injected STZ was lower as compared to HFD mice without STZ injection.

The above results showed that in the ND+STZ mice there are not significant changes in blood fasting glucose, plasma insulin and lipid parameters, such as TC, TG, HDL, and PI, in comparison with untreated ND fed mice. Therefore, obesity and insulin resistance were the important causes of diabetes.

3.3. Effect of the extract fractions on blood fasting glucose level and plasma insulin secretion in type 2-diabetic mice

Table 3. Effect of extract fractions on blood fasting glucose and plasma insulin secretion in obese-diabetic mice

Treatment with extract fraction	Glucose (mmol/l)		Change of glucose (%)	Plasma insulin (pmol/l)		Change of insulin (%)
	Starting point	Final		Starting point	Final	
Obese -diabetic mice untreated	23.14±0.57	23.20±0.70	0	245.87±9.10	235.65±3.47	↓4.15
Obese -diabetic mice + EtOH	23.34±0.36	17.58±0.35*	↓24.68	237.63±5.63	270.71±3.63*	↑13.92
Obese -diabetic mice + Chlf	23.24±0.64	19.45±0.75*	↓16.31	235.75±4.32	248.79±8.28*	↑5.53
Obese -diabetic mice + EtOAc	23.78±0.52	15.04±0.42*	↓36.75	245.87±9.72	309.16±2.57	↑25.74
Obese -diabetic mice + Metformin	23.14±0.57	11.75±0.20*	↓49.22	238.20±2.72	267.55±4.91*	↑12.32

Values are mean SEM, *: $p < 0.05$ vs. starting point

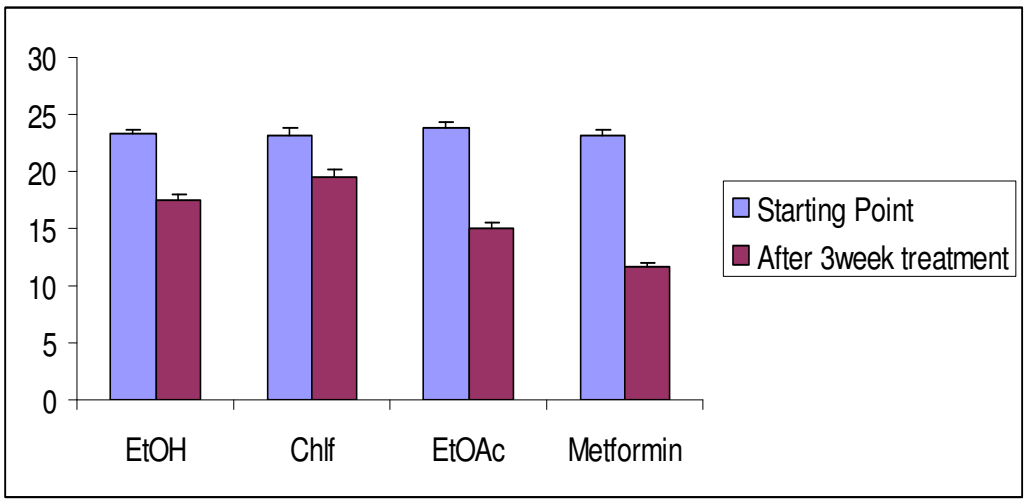

Fig. 2. Effect of extract fractions from Ipomoea batatas leaves on blood fasting glucose of type 2- diabetic mice. EtOH: ethanol extract concentrate; Chlf: Chloroform extract concentrate; EtOAc: ethyl acetate extract concentrate (↓ :decrease).

The obtained results indicated that the highest hypoglycaemic effect of ethyl acetate fraction with dose of 1000mg/kg is 36.75% (fig.2) compared to the mice before treatment (table 3). Moreover, stimulating effect of insulin secretion was proven. Especially, insulin secretion stimulating effect of the EtOAc fraction was 25.74%, higher as compared to other fractions.

3.4. Effect of ethyl acetate fraction on lipid parameters of obese-diabetic mice

Table 4. Hypolipidemic effect of ethyl acetate. fraction on lipid parameters of diabetic mice. The results indicated that ethyl acetate extract possessed hypolipidemic effect in obese-diabetic mice

	Mice before treatment	Mice after 3 weeks treatment	Changes (%)
TC (mg/dl)	258.13 ± 15.23	167.31 ± 14.11	↓35.18
TG (mg/dl)	642.86 ± 10.42	455.35 ± 9.6	↓29.17
HDL-c (mg/dL)	13.57 ± 2.73	20.53 ± 1.52	↑51.28
LDL-c (mg/dL)	115.00 ± 7.61	55.71 ± 6.17	↓51.55

3.5 .Effect of ethyl acetate extract fraction on metabolic enzymes

The above results showed that the ethyl acetate fraction possessed the highest hypoglycaemic effect in diabetic mice. Following, we continued to assess the effect of this fraction on some metabolic enzymes, such as hexokinase and glucose-6-phosphatase. The obtained results were presented in fig 3.

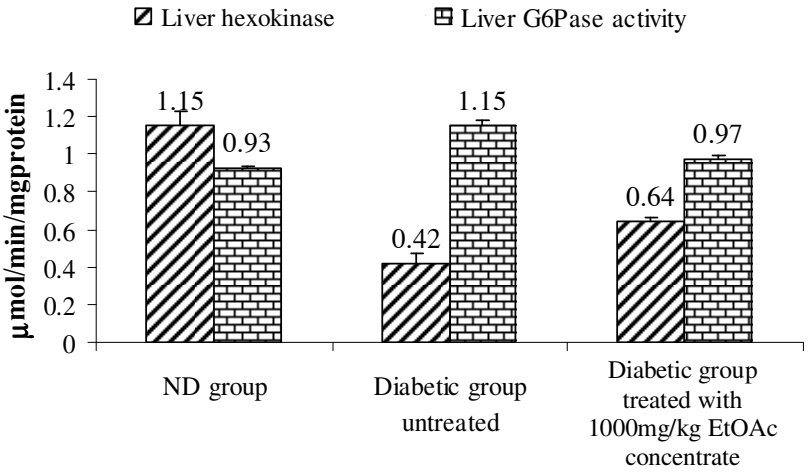

Fig. 3. Effect of ethyl acetate fraction on hexokinase and glucose-6-phosphatase activity (Enzymatic activity was identified as mmol/min/mg protein).

Our results showed that there is difference between hexokinase and glucose-6-phosphatase activity of normal mice and diabetic mice. In normal mice, the hexokinase activity than G6Pase activity. While, in diabetic mice, hexokinase activity was decreased and glucose-6-phosphatase activity was increased, 63.48 % decrease and 23.66% increase respectively

The ethyl acetate fraction with dose of 1000mg/kg increased significantly hexokinase activity and reduced significantly glucose-6-phosphatase activity, namely by 52.38% and 15.65% respectively in treated diabetic mice.

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Tác dụng của dịch chiết lá khoai lang (*Ipomoea batatas (L.)* lam) lên sự giảm đường huyết, sự tiết insulin máu và trên các enzym chủ chốt của trao đổi Carbohydrat ở chuột béo phì và đái tháo đường thực nghiêm

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Tác dụng hạ lipid máu và hạ đường huyết của phân đoạn dịch chiết ethyl acetate từ lá khoai lang (*Ipomoea batatas*(L.) Lam) họ Bìm bìm (*Convolvulaceae)* đã được chứng minh ở chuột thực nghiệm béo phì và đái tháo đường typ2 (ĐTĐ). Khi chuột béo phì và ĐTĐ typ2 được điều trị hằng ngày bằng đường uống với liều 1000mg/kg bột dịch chiết ethyl acetate đông khô trong 21ngày (3tuần), chúng tôi đã chỉ rõ tác động làm giảm cao nhất đường huyết (36,77%) và mỡ máu như Cholesterol (35,18%), Triglycerid (29,17%), LDLc(51,97%) ở chuột béo phì và giảm ĐTĐ typ2 đã được điều trị so với kiểm tra. Cơ chế hoạt động làm giảm đường huyết của phân đoạn dịch chiết ethyl acetate từ lá khoai lang đượoc chứng minh là do dịch chiết đã tăng cường sự hoạt động của enzym hexokinase, kích thích sự bài tiết insulin trong máu và kìm hãm hoạt động của enzym tân tạo glucose là Glucose 6 photphatase ở gan.