

Hypoglycemic and Antihyperlipidemic Effect of Four Korean Medicinal Plants in Alloxan Induced Diabetic Rats

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Abstract: To consider potentially new hypoglycemic and antihyperlipidemic sources, aqueous extracts from four Korean medicinal plants, *Chrysanthemum coronarium*, *Dioscorea batatas*, *Morus alba*, *Citrus unshiu*, were investigated in alloxan-induced diabetic rats. To induce diabetes, 36 rats were administered alloxan orally (120 mg/kg body weight) for 2 weeks and among those 6 rats were used as a diabetic control. Effect of extracts from four medicinal plants on blood glucose levels of diabetic rats was determined at various time interval for 9 h after oral administration of the first extract at 100 mg dose /kg body weight. In addition, GOT, GPT, LDH, total cholesterol (TC) and triglyceride (TG) were determined 7 days after daily oral administration of each extract. The glucose levels of diabetic rats treated with *C. coronarium* and *M. alba* were significantly decreased, respectively, at 3 h and 5 h after administration ($p < 0.05$). The enzyme activities of GOT, GPT and LDH of diabetic rats were also decreased in diabetic rats treated with four plant extracts respectively. In particular, the administration of extract from *M. alba* tends to bring the GOT, GPT and LDH values of diabetic rats to near normal. There was no significant difference in TC between diabetic control and diabetic rats treated, respectively, with extracts of *C. coronarium*, *D. batatas* and *M. alba*, except *C.unshiu* extract showing a significant decrease when compared to the diabetic control. The TC value was brought down by 33.3% fall from the value of diabetic rats, which shows more decrease than that of glibenclamide-treated ones. There was a significant difference in TG between the diabetic control and diabetic rats treated, respectively, with extracts of *C. coronarium*, *D. batatas*, *M. alba* and *C.unshiu*. The results suggest that the administration of *C. coronarium* and *M. alba* have a hypoglycemic effect in diabetic rats and their effect was equivalent to that of glibenclamide. The administration of *C.unshiu* shows more antihyperlipidemic effect than antidiabetic effect.

Key words: Hypoglycemic, antihyperlipidemic, *Chrysanthemum coronarium*, *Dioscorea batatas*, *Morus alba*, *Citrus unshiu*, alloxan-induced diabetic rats

INTRODUCTION

Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein^[1]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs^[2]. In diabetic rats, the utilization of impaired carbohydrate leads to accelerate lipolysis, resulted in hyperlipidemia^[3,4]. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies^[5-10]. Antihyperglycemic effects of these plants are attributed to their ability to restore the function of

pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature^[11,12], however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having antidiabetic effect^[13].

Chrysanthemum coronarium L. is regarded in East Asia as a health vegetable and folk medicine. The leaves of *C. coronarium* have been shown to be rich in quercetin and its glycosides, rutin and isoquercetin^[14]. *Dioscorea batatas* (Chinese yam) is a sweet soothing herb that stimulates the stomach and spleen and has a tonic effect on the lungs, liver and kidneys. Yam extract

has proved to be helpful as a digestion-aiding agent for patients suffering from hyperglycemia or hyperlipidemia^[15,16]. The various parts of the mulberry tree (*Morus alba* L.) have been applied in the clinical treatment of various diseases in Oriental medicine. Recent evidence shows that the leaves and shoots from the mulberry tree possess several medicinal properties, including hypoglycemic, hypotensive and diuretic effects. In addition, it has been demonstrated that *M. alba* is clinically effective in the treatment and prevention of diabetes^[17]. The dried tangerine (*Citrus unshiu* Markovich) peel contains plenty of human indispensable nutrients, such as protein, vitamin C, carotenoid and life supporting trace elements^[18]. Hence, in the present study the aqueous extracts of four Korean diet plants, *C. coronarium*, *D. batatas*, *M. alba* and *C. unshiu* were evaluated for the potential antidiabetic and antihyperlipidemic effect on alloxan-induced diabetic rats and to compare the effect with glibenclamide. To avoid the risk of serious complications from diabetes, such as heart and blood vessel diseases, controlling not only blood glucose levels but also lipid levels are necessary^[19]. Therefore, we determined the effect of aqueous extracts from four plants on glucose, GOT, GPT, LDH, total cholesterol (TC) and triglyceride (TG) in the blood.

MATERIALS AND METHODS

Preparation of plants extract and chemicals: *C. coronarium*, *D. batatas* and *C. unshiu* were purchased from local market and *M. alba* was a gift from the Silk Yarn Farm, Daejeon, Korea (Table 1). Two hundred gram of each plant was extracted individually and decocted in a round flask with distilled water (3000 mL) at 100°C for 1 h. The aqueous extracts were subsequently filtered, evaporated in a rotavapor at 40-50°C under reduced pressure and freeze-dried as a powder. Alloxan was purchased from Sigma Chemical Company Inc. the other biochemicals used in this experiment were purchased from Stanbio Laboratory.

Experimental animals and alloxan-induction of experimental diabetes: Male SD rats weighing 180-200 g were obtained from the Korea Research Institute of Chemical Technology. The rats were housed in polycarbonated cages at a temperature regulated (22°C) and humidity (55%) controlled room with a 12 h light/12 h dark cycle. A water and standard pellet diet were available *adlibitum* throughout the experimental period. The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 120 mg kg⁻¹ b.wt. Two weeks after treatment, rats with moderate diabetes having glycosuria (indicated by Benedict's qualitative test)

and hyperglycemia (i.e. with a blood glucose of 200-300 mg dL⁻¹) were used for the experiment. The schedules and procedures were performed in the Experimental Animal Handling Facility in Department of Biology & Medicinal Science at Pai Chai University, Daejeon, Korea, in compliance with ethical regulations.

Extracts and drug administration: Each extract was suspended in distilled water and administered orally through an intragastric tube at a dose of 100 mg kg⁻¹ b.wt. Glibenclamide was administered at a dose of 0.2 mg kg⁻¹ b.wt.

Experimental design: In the experiment, a total of 42 rats (36 diabetic surviving rats, 6 normal rats) were used. The rats were divided into seven groups of six rats each after the induction of alloxan diabetes. Group 1: normal rats. Group 2: diabetic control rats. Group 3: diabetic rats given extract of *C. coronarium*. Group 4: diabetic rats given extract of *D. batatas*. Group 5: diabetic rats given extract of *M. alba*. Group 6: diabetic rats given extract of *C. unshiu*. Group 7: diabetic rats given glibenclamide. An oral administration was conducted with each extract with 100 mg kg⁻¹ b.wt. Blood glucose was measured at 1, 3, 5, 7 and 9 h after first extract administration. Blood was withdrawn from the tail vein each time. To measure lipids and some enzymes, oral administration was conducted daily for 7 days. At the end of 7 days, all the rats were anaesthetized by pentobarbitone sodium (60 mg kg⁻¹) and opened at the abdomen. Blood was withdrawn from the abdominal aorta and centrifuged at 3000 rpm for 10 min to obtain the plasma.

Biochemical analysis: Blood glucose was determined by the O-toluidine method. GOT (Glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transaminase), LDH (Locate dehydronase), TC (total cholesterol) and TG (triglyceride) were measured using Stanbio Laboratory auto reagents on a photometer CH-100 plus (Dae Guang meditec. Ltd.). GOT and GPT were estimated by the method of Neeley *et al.*^[20] using NADH oxidation reaction. The serum (0.1 mL) was added to 1 mL of auto reagent and incubated at 37°C for 1min. The absorbance was measured at 340 nm and the values were expressed as unit L⁻¹. LDH was determined by the method of Mercer^[21] using Wacker method. To 0.025 mL of the serum, 1 mL of auto reagent was added and incubated at 37°C for 1 min. The absorbance was measured at 340 nm and the value was expressed as unit L⁻¹. TC was estimated by the method of Savoldi *et al.*^[22] using modified Trinder method. The serum (0.01 mL) was added to 1 mL of auto reagent and incubated at 37°C for 5 min, the absorbance was measured at 500 nm. The content was expressed as mg dL⁻¹. TG was determined by the method of Chan *et al.*^[23] using glyceryl phoshate oxidase method.

Table 1: Plants used for the present experiment

Botanical name	Common name	Family	Part used
<i>Chrysanthemum coronarium</i>	Crown daisy	Compositae	Leaves
<i>Dioscorea batatas</i>	Yam	Dioscoreaceae	Root
<i>Morus alba</i>	Mulberry	Moraceae	Leaves
<i>Citrus unshiu</i>	Dried Tangerine Peel	Rutaceae	Pericarpium

Table 2: Effect of plant extracts on plasma glucose levels in alloxan-induced diabetic rats (mg dL⁻¹)

Treatment	0 h	1 h	3 h	5 h	7 h	9 h
Normal	94±3.6	135±7.1	128±6.8	117±5.9	112±3.2	119±6.4
Diabetic control	464±20.8	440±73.1	500±48.2	461±60.2	507±30.6	553±23.5
+ <i>C. coronarium</i>	416±19.8	367±3.6 ^a	338±9.5 ^a	346±4.4 ^a	411±15.0	411±15.1
+ <i>D. batatas</i>	556±13.5	≥600±0.0 ^b	≥600±0.0 ^b	≥600±0.0 ^b	597±1.1	589±5.1
+ <i>M. alba</i>	566±15.7	417±31.1	375±24.4 ^a	338±26.3 ^a	381±29.1 ^a	370±40.7 ^a
+ <i>C. unshiu</i>	225±8.6	237±5.8	255±12.9	331±9.9 ^a	318±5.5 ^a	385±0.8 ^a
+ Glibenclamide	541±20.5	477±32.4	417±18.2	386±18.1	363±22.2	346±22.0

Values are mean concentration of blood glucose ± S.E. (n=6).

^aSignificantly increased or decreased values compared with 0 h data (p<0.05).

^bNo measurement by the device due to blood glucose level over 600 mg dL⁻¹.

To 0.01 mL of the serum, 1mL of auto reagent was added and incubated at 37°C for 5 min, the absorbance was measured at 500 nm. The content was expressed as mg dL⁻¹.

Statistical analysis: All data were expressed as means ± S.E. Significant differences among the groups were determined by one-way analysis of variance using the SPSS statistical analysis program. Statistical significance was considered at p<0.05.

RESULTS

Effect of experimental plants on plasma glucose levels: The effect of aqueous extracts from four medicinal plants on the blood glucose levels of experimental animals was determined at various time interval for 9 h after oral administration at 100 mg dose kg⁻¹ b.wt. (Fig. 1 and Table 2). There was a significant elevation in the blood glucose level by 3.3-5 times during experimental time period in alloxan-induced diabetic rats, when compared to normal rats. The administration of *C. coronarium* extract caused the blood glucose levels of diabetic rats to 83.4, 67.6, 75.1, 81.1 and 74.3% at the time interval of 1, 3, 5, 7 and 9 h, respectively (p<0.05). Maximum reduction of 32.4% was observed 3 h after treatment. The administration of *M. alba* extract produced the most significant reduction (p<0.05) among four medicinal plants in the blood glucose levels of 34, 41, 33 and 35% at 3, 5, 7 and 9 h respectively. On the other hand, the administration of *C. unshiu* extract showed a significant increase of 171% after 9 h (p<0.05). In the case of *D. batatas*, it could not be measured because the device could not measure blood glucose over 600 mg dL⁻¹.

Effect of experimental plants on plasma GOT, GPT and LDH: Table 3 shows the activities of GOT, GPT and LDH of experimental rats. Compared with normal rats, diabetic rats showed significantly more activities of serum GOT, GPT and LDH by 3.9, 2.6 and 27.1 times, respectively. Treatment with all experimental plant extracts significantly reduced the activity of GOT, GPT and LDH in the diabetic control rats (p<0.05). The administration of *C. coronarium* extract brought down GOT, GPT and LDH values in alloxan-induced diabetic rats to 52.8, 85.7 and 6.5%, respectively. The administration of *D. batatas* extract brought down GOT, GPT and LDH values in alloxan-induced diabetic rats to 30.2, 39.8 and 27.2%, respectively. The administration of *M. alba* extract tends to bring the GOT, GPT and LDH values of alloxan-induced diabetic rats to near normal. The administration of *C. unshiu* extract brought down GOT, GPT and LDH values in alloxan-induced diabetic rats to 31.6, 51.6 and 6.5% respectively.

Effect of experimental plants on plasma lipid contents: While no changes were seen in TC levels between normal group and diabetic control, a significant difference in TG was observed in plasma TG levels between normal group and diabetic control (Table 4). TG level of diabetic control was increased by 3 times from that of normal ones on 7 days post hyperlipidemia induction. There was no significant difference in TC between diabetic control and diabetic groups treated, respectively, with aqueous extracts of *C. coronarium*, *D. batatas* and *M. alba*. However, in diabetic rats treated with *C. unshiu* extract there was a significant decrease in TC when compared to the diabetic control.

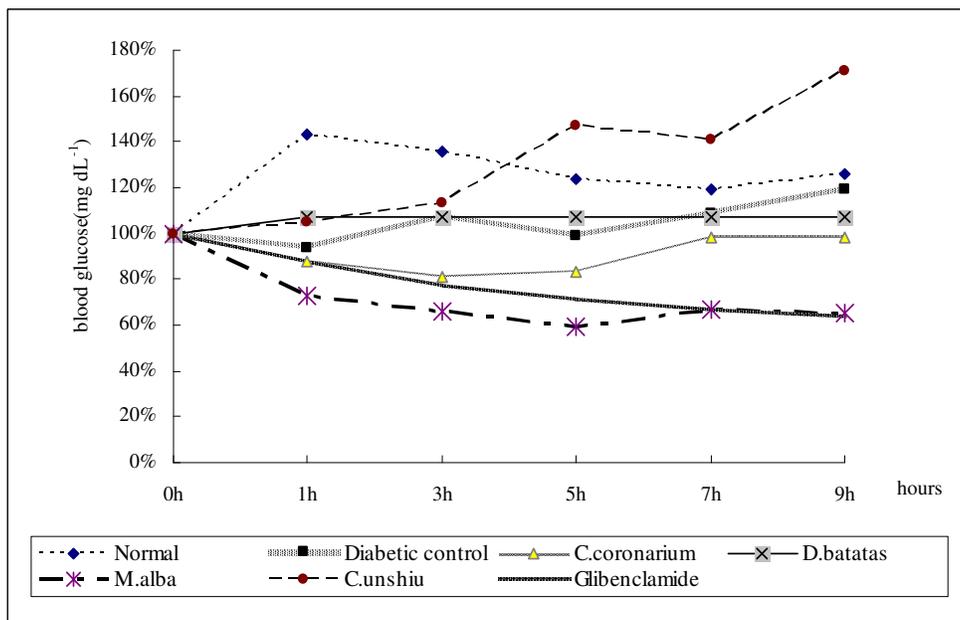


Fig. 1: Percentage of effect of experimental plants on plasma glucose levels compared with 0 h data in alloxan-induced diabetic rats (%). Values are mean percent of blood glucose concentration (n= 6)

Table 3: Effect of plant extracts on GOT, GPT and LDH in alloxan-induced diabetic rats (unit L⁻¹)

Group	GOT	GPT	LDH
Normal	74±3.0	63±4.3	43±8.1
Diabetic control	288±2.1	161±2.7	1167±24.9
+ <i>C.coronarium</i>	152±4.7 ^a	138±2.7 ^a	76±2.4 ^a
+ <i>D.batatas</i>	87±4.1 ^a	64±3.2 ^a	317±2.4 ^a
+ <i>M.alba</i>	69±14.0 ^a	70±6.0 ^a	41±10.3 ^a
+ <i>C.unshiu</i>	91±2.4 ^a	83±3.8 ^a	76±13.5 ^a
+ Glibenclamide	104±11.3 ^a	79±6.5 ^a	256±40.5 ^a

Values are mean activities of GOT, GPT and LDH ± S.E. (n=6).

^aStatistically significant compared with diabetic control by t-test at p<0.05.

Table 4: Effect of experimental plants on lipid contents in alloxan-induced diabetic rats (mg dL⁻¹)

Group	Total cholesterol	Triglyceride
Normal	62±6.6	83±5.2
Diabetic control	76±4.4	262±5.7
+ <i>C.coronarium</i>	90±2.7	156±2.5 ^a
+ <i>D.batatas</i>	72±2.8	66±0.3 ^a
+ <i>M.alba</i>	72±2.4	105±12.1 ^a
+ <i>C.unshiu</i>	58±3.0 ^a	58±2.7 ^a
+ Glibenclamide	64±4.6	100±5.4 ^a

Values are mean concentration ± S.E. (n=6).

^aStatistically significant compared with diabetic control by t-test at p<0.05.

Its TC level was brought down by 33.3% fall from TC of diabetic control, which is more decrease than that of glibenclamide-treated ones. There was a significant difference in TG between the diabetic control and diabetic rats treated, respectively, with extracts of *C.*

coronarium, *D. batatas*, *M. alba* and *C. unshiu*. The reduced level of each extract was 40.5, 74.8, 59.9 and 77.9% respectively. In particular, the values of *D. batatas* and *C. unshiu* treated diabetic rats were less than those of normal rats and glibenclamide-treated diabetic rats.

DISCUSSION

Alloxan induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic β -cell, resulting in a decrease in endogenous insulin release^[24,25]. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in alloxan-induced diabetic animals^[26-36]. In the present study, the aqueous extracts of *C. coronarium* and *M. alba* effectively decreased the blood glucose in alloxan-induced diabetic rats, which is

even better than glibenclamide. The result was in collaborative with Kim *et. al.*^[17] who reported that *M. alba* proves to aid for the recovery from the central nervous system complications of diabetes mellitus and in controlling the desire for food under diabetic conditions. However, the mechanism of these plants used has not been clearly defined. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver cell damage. The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels and secondarily due to the effects of the diabetogenic agent alloxan^[37]. *C. coronarium* contains chlorogenic acid and its related compounds, 3,5-dicaffeoylquinic acid (SP-1) and 4-succinyl-3,5-dicaffeoylquinic acid (SP-2), known as its major antioxidants^[38]. In our previous study, *M. alba* extract showed strong free radical scavenging and antioxidant activities and also showed a protective effect on DNA damage caused by hydroxyl radicals^[39]. Based on above-mentioned reports, we suggest that the possible mechanism of action by aqueous extracts from *C. coronarium* and *M. alba* could be related to antioxidants that aid to recover from impaired metabolism of glucose. Previous studies have demonstrated that *D. batatas* may prove helpful as a digestion-aiding agent for patients suffering from hyperglycemia^[40] or hyperlipidemia^[41], but we have experienced a negative result regarding the hypoglycemic effect.

The activities of GOT and GPT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. Therefore, we used the activities of GOT, GPT and LDH in the circulation as indicators of hepatic damage. In the present study, all treatment groups with experimental plant extracts effectively reduced plasma GOT, GPT and LDH activities in diabetic rats, suggesting that the aqueous extracts of experimental plants may prevent hepatic injury associated with diabetes.

The increases in plasma lipid, TC and TG levels occur in diabetes, which is related with significant changes in lipid metabolism and structure^[42]. Although abnormalities in cellular cholesterol metabolism could be partly responsible for the changes in the plasma cholesterol levels in diabetes, the precise mechanisms underlying these enzymatic changes have yet to be elucidated^[43, 44]. In the present study, TC value of diabetic rats treated with *C. unshiu* extract was significantly lower than those treated with other plant extracts. It is known as an herb effective in removing dampness-phlegm, one of symptoms in diabetes based on Korean and Chinese medicine. Diabetes may be induced by excessive consumption of alcohol or fatty,

sweet, pungent or fried foods. The excess fat transforms into interior dampness-phlegm, accumulates and impairs yin fluid and thereby prevents food essence from nourishing the muscles, skin, lungs, liver, kidney and stomach^[45]. Several plant constituents are known to reduce TG^[46] which is usually increased in the serum of diabetes^[47]. Such a significant increase in TG may be due to the lack of insulin under diabetic condition, while insulin activates the enzyme lipoprotein lipase and hydrolysis TG under normal condition. In the present study, the administration of aqueous extracts from all experimental plants and glibenclamide effectively reduced TG in alloxan-induced diabetic rats.

On the basis of the aforementioned results, we concluded that *C. coronarium* and *M. alba* have a significant hypoglycemic effect in diabetic rats and that their effect was comparable to that of Glibenclamide. Therefore, these medicinal plants are considered to be effective and alternative treatment for diabetes. On the other hand, *C. unshiu* has a greater antihyperlipidemic effect than antidiabetic effect; thereby *C. unshiu* and *D. batatas* are not suitable for use as antidiabetic drugs.

ACKNOWLEDGEMENTS

This work was supported by a grant from Ministry of Commerce, Industry and Energy (MOCIE) through the Research Center for Bio-Medicinal Resources (Bio-Med RRC) at Pai Chai University.

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