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Effects of *Taraxacum mongolicum* Extract on Blood Glucose Levels and Lipid Profiles in Streptozotocin-Induced Diabetic Rats

Hye Kyoung Han and †Eun Young Choi*

Visiting Professor, Dept. of Food and Nutrition, KyungBuk College, YeongJu 36133, Korea
**Assistant Professor, Dept. of Food and Nutrition, Bucheon University, Bucheon 14632, Korea*

Abstract

This study was designed to evaluate antihyperglycemic and antihyperlipidemic effects of ethanol extracts of *Taraxacum mongolicum* (*T.m.*) on streptozotocin (STZ)-induced diabetic rats. Sprague-Dawley rats were randomly assigned to five groups: normal (NC), STZ-control (DC), and three experimental groups. Diabetes was induced in Sprague-Dawley rats with a single intravenous injection [45 mg/kg body weight (b.w.)] of STZ. An ethanol extract of *T.m.* was orally given to diabetic rats for 14 days. Three experimental groups were additionally treated with *T.m.* extract at doses of 1 g/kg b.w./day for *T.m.*-1, 2 g/kg b.w./day for *T.m.*-2, and 3 g/kg b.w./day for *T.m.*-3. Oral administration of *T.m.*-2 significantly increased their body weights. *T.m.*-1 and *T.m.*-2 significantly decreased aspartate aminotransferase (AST) levels than DC. *T.m.*-1 and *T.m.*-2 group significantly decreased blood glucose levels. Total cholesterol, triglycerides, and free fatty acids were significantly decreased whereas high-density lipoprotein cholesterol was significantly increased in groups treated with *T.m.* extract than those in the DC group. These results support the fact that administration of *T.m.* extract can reduce hyperglycemia and hyperlipidemia risk in diabetic rats.

Key words: *Taraxacum mongolicum*, streptozotocin-diabetic rats, blood glucose, lipid profile

Introduction

The genus *Taraxacum* is a member of the family *Asteraceae,* which is widely distributed in warm temperature zones of the northern hemisphere, especially Japan, China, and Korea, where it has many biological activities. *Taraxacum* species have attracted the attention of researchers because of their anti-inflammatory, anti-carcinogenic, anti-allergic, anti-hyperglycemic, anti-coagulatory, and analgesic activities (Shi et al. 2008). *Taraxacum mongolicum* (*T.m.*) is a famous traditional medicine that is frequently used to treat inflammatory disorders and viral infectious diseases (Ma et al. 2019). Active compounds of *T.m.* include flavonoids, polysaccharides, phenolic acids, sesquiterpene lactones, triterpenoids, tocopherols, pigments, vitamins, amino acids, coumarins, and sterols that possess anti-inflammation, anti-cancer, anti-oxidation, anti-thrombosis, anti-hypoglycemia, immunity-enhancing, liver and gallbladder protecting, and other pharmacological effects (Zeng et al. 2018; Zhang et al. 2022). However, translational data about the effect of *T.m.* on the pathogenesis of diseases are lacking.

Due to lifestyle changes and aging population, the number of diabetes population continues to increase worldwide. The global diabetic population in 2014 was approximately 422 million, accounting for 8.5% of the adult population. It has been estimated to increase to 783 million by 2045 (Saeedi et al. 2019). In Korea, the proportion of diabetic subjects aged 30 years or older had increased from 11.6% in 2011 to 13.6% in 2020 (Kim et al. 2021). Diabetes is a chronic disease caused by high blood glucose levels. There is no known cure for diabetes. It can be controlled. In some minor cases, it may go into remission (Kang et al. 2008; Lee NY 2013; Han & Choi 2020). Uncontrolled glucose levels might result in various complications such as heart diseases and stroke (Heather & Clarke 2011). Han et al. (2009) have reported that the intake of *T.m.* powder can reduce blood glucose, triglycerides (TG) and free fatty acids (FFA) in diabetic

† Corresponding author: Eun Young Choi, Assistant Professor, Dept. of Food and Nutrition, Bucheon University, Bucheon 14632, Korea. Tel: +82-32-610-3442, Fax: +82-32-610-3205, E-mail: eychoi@bc.ac.kr

rats Therefore, the present study was aimed to investigate antidiabetic and antihyperlipidemic potential of ethanol extracts of *T.m.* in different concentrations in streptozotocin (STZ)-induced diabetic rats. However, whether *T.m.* ethanol extract might possess antihyperglycemic and antihyperlipidemic effects on diabetes remains unclear. Therefore, the aim of this study was to investigate antidiabetic and antihyperlipidemic effects of ethanol extracts of *T.m.* at different concentrations on STZ-induced diabetic rats.

Materials and Methods

1. Experimental materials and extraction methods

T.m. plants were shade dried, powdered, and extracted three times with 80% ethanol using a Soxhlet apparatus for 4 hours. The ethanol extract was concentrated in a rotary evaporator (SunilEyela Co., Ltd., Seongnam, Korea) at 35~40℃ under reduced pressure. The ethanol extract of *T.m.* was stored at 2~8℃ until completion of pharmacological studies.

2. Animal care and experimental diet

Sprague-Dawley male rats weighing about 220 g were obtained from Samtaco (Sam: TacN (SD) BR, Osan, Korea) and pre-fed with pallet (Feedlap, Guri, Korea) for one week to adapt to the environment. These rats were all individually kept in stainless steel cages in an air-conditioned room with controlled temperature (20~22℃) and automatic lighting (alternation 12 hours periods of light and dark). They were then divided into two groups: a normal control (NC) group (n=6) and a diabetes-induced group (n=24). Those in the diabetes-induced group were then assigned to four groups with a weight-based randomized block design: a diabetes control group (DC) and three experimental groups (*T.m.*-1, experimental diabetic group treated with ethanol extract of *T.m.* 1 g/kg b.w./day; *T.m.*-2, experimental diabetic group treated with ethanol extract of *T.m.* 2 g/kg b.w./day; and *T.m.*-3, experimental diabetic group treated with ethanol extract of *T.m.* 3 g/kg b.w./day). Rats in all groups were supplied with the AIN-93 formula (Reeves PG 1997) as an experimental formula (Table 1). They had free access to food and water during the experimental period. *T.m.* extracts were suspended in water with Tween 80 and administered orally at a dose for 14 days. NC ad DC groups were given the same volume of a 5% Tween 80 solution. Rats were individually kept in stainless steel cages for 14 days. This animal experiment was conducted in accordance with guidelines provided by the Institutional Review Board (IRB) and Institutional Animal Experimentation Ethics Committee of Duksung Women's University (2022-011-021).

Table 1. Composition of AIN-93 diets (g/kg diet)

Components	AIN-93 diet
Corn starch	465.692
Casein	140.000
Dextrinized corn starch	155.000
Sucrose	100.000
Soybean oil	40.000
Fiber	50.000
Mineral mix[1)]	35.000
Vitamin mix[2)]	10.000
L-Cystine	1.800
Choline bitartrate	2.500
Tert-Butylhydroquinone	0.008

[1)] AIN-93 mineral mixture.
[2)] AIN-93 vitamin mixture.

3. Induction of diabetes

Diabetes was induced in 16-hour fasted rats by a single intravenous injection of STZ (Sigma-Aldrich Chemical Co., St Louis, MO, USA) at a dose of 45 mg/kg b.w. in 0.1 M cold citrate buffer (pH 4.5) (Lee & Kim 1999). The same amount of citrate buffer solution was injected into the NC group. At 24 hours after injection, blood was collected from the eye vein, centrifuged, and taken to measure blood glucose. Diabetes was considered for animals with plasma glucose concentrations of 300 mg/dL or more.

4. Sample collection and biochemical analysis

To examine changes in blood glucose (Raabo & Terkildsen 1960) and cholesterol levels on the 0th, 4th, 7th, and 11th days of the experiment, experimental animals were anesthetized with ether. Blood was collected from an eye vein and centrifuged at 3,000 rpm for 15 minutes (HA 300, Hanil Centrifuge Co., Ltd., Seoul, Korea). Plasma was taken to measure glucose and total cholesterol levels. On the 14th day of the experiment, blood samples were taken into heparinized tubes immediately after anesthetizing experimental animals with ether. Animals were then sacrificed with a guillotine. Blood samples were centrifuged

at 3,000 rpm for 15 minutes. Only plasma was taken and used as an analysis sample. Hematocrit (Bauer JD 1982) was measured immediately after blood collection. Body weight and food intake were measured at regular time points for 14 days. Food efficiency ratio (FER) was calculated by dividing the weight gain during the total breeding period by the food intake during the same period. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using a colorimetric assay (Reitman & Frankel 1957) with commercially available kits (Embiel Ltd., Gunpo, Korea). The absorbance was read at 505 nm according to the manufacturer's protocols. Plasma concentrations of TG (Embiel Ltd.), total cholesterol (TC) (Embiel Ltd.), high-density lipoprotein cholesterol (HDL-chol) (Embiel Ltd.), and FFA (Shinyang Chemical Ltd., Seoul, Korea) were quantified by spectrophotometry using commercially available kits. TG levels were determined using a lipase-glycerol phosphate assay (Giegel et al. 1975). Cholesterol measurements were based on the cholesterol oxidase method (Richmond W 1973). HDL-chol was determined after separating the HDL fraction using a heparin-manganese precipitation procedure (Finley et al. 1978).

5. Statistical analysis

All statistical analyses were conducted using SPSS (Statistical Package for the Social Science Ver. 23.0, SPSS Inc., Chicago, IL, USA). Values are presented as means and standard deviation (S.D.). One-way analysis of variance (ANOVA) followed by a Duncan's multiple range test was used to determine differences between means of experimental groups. A p-value<0.05 was considered significant.

Results and Discussion

1. Effects of *T.m.* ethanol extract on body weight, food intake, and food efficiency ratio

Body weights of rats in diabetic induced groups after treatment with *T.m.* ethanol extract for 14 days were significantly lower than those in the control group (Table 2). Body weights of race in the *T.m.*-2 group were higher than those in other diabetic-induced experimental groups. Table 3 presents effects of *T.m.* ethanol extract on food intake and food efficiency ratio (FER). All diabetes groups had significantly higher average food intake than the NC group. Among diabetic groups, there were no significant differences in food intake. Diabetic rats had higher dietary intake than the control group due to an increase in neuropeptide Y (NPY) mRNA in insulin-deficient rat and a decrease in the action of leptin receptors in the hypothalamus. It has been reported that dietary intake is decreased when insulin is replenished (Clark et al. 2004). All diabetes-induced groups had significantly lower FER than the control group (Table 3). This might be due to degenerative metabolism caused by diabetes as a cause of continuous weight loss, although diabetes-induced groups had higher dietary intake than the normal group (Sun et al. 2023). *T.m.*-2 showed the highest level of weight gain among diabetes-induced groups. *T.m.*-2 might have suppressed weight loss due to diabetes induction. In other words, *T.m.*-2 administration could recover degenerative changes such as weight loss due to diabetes induction to some extent.

Table 2. Initial and final body weight of normal and diabetic rats fed extract of *Taraxacum mongolicum*[1)]

Group[2)]	Initial b.w.[3)] (g)	Final b.w. (g)	Weight gain (g/14 day)
NC	248.9±7.1[NS4)]	308.8±17.7[c5)]	59.8±22.1[c]
DC	250.7±10.5	234.8±19.5[a]	−15.8±12.8[a]
T.m.-1	251.1±6.8	237.8±12.7[a]	−13.3±13.8[a]
T.m.-2	257.1±12.2	269.6±28.3[b]	12.5±19.7[b]
T.m.-3	251.0±7.4	233.5±25.7[a]	−17.5±22.5[a]

1) Values are mean±S.D. of 6 rats.
2) NC, normal control; DC, diabetic, untreated; *T.m.*-1, diabetic treated with ethanolic extract of *Taraxacum mongolicum* (1 g/kg b.w.); *T.m.*-2, diabetic treated with ethanolic extract of *Taraxacum mongolicum* (2 g/kg b.w.); *T.m.*-3, diabetic treated with ethanolic extract of *Taraxacum mongolicum* (3 g/kg b.w.).
3) b.w.: body weight.
4) NS: not significant at p<0.05.
5) Values with different superscript within the same column are significantly different at p<0.05 by Duncan's multiple range test.

Table 3. Food intake and food efficiency ratio (FER) of normal and diabetic rats fed extract of *Taraxacum mongolicum*[1)]

Group[2)]	Food intake (g/day)			FER[4)]
	1 wk	2 wk	Mean	
NC	25.5±4.2[a3)]	25.4±3.6[a]	25.4±3.4[a]	0.158±0.020[b]
DC	32.2±4.6[bc]	44.9±8.5[b]	38.5±6.3[b]	−0.028±0.022[a]
T.m.-1	30.7±6.0[ab]	44.6±3.3[b]	37.7±4.6[b]	−0.033±0.014[a]
T.m.-2	37.1±3.0[c]	46.0±7.6[b]	41.6±4.1[b]	−0.014±0.022[a]
T.m.-3	30.1±3.1[ab]	43.4±6.0[b]	36.8±3.4[b]	−0.036±0.047[a]

1) Values are mean±S.D. of 6 rats.
2) See the legend of Table 2.
3) Values with different superscript within the same column are significantly different at $p<0.05$ by Duncan's multiple range test.
4) FER: body weight gain/food intake.

2. Effects of *T.m.* ethanol extract on hematocrit and plasma aminotransferase activity

After 14 days of treatment with *T.m.* ethanol extract, hematocrit levels were not significantly different between the control group and experimental groups (Table 4). Dai & McNeill (1994) have also reported no significant difference in hematocrit between normal and diabetic rats. It has been suggested that the increase in hematocrit in diabetic rat compared to normal is due to water loss in blood caused by polyuria (Koh et al. 1999). Plasma ALT and AST activities are presented in Table 4. ALT and AST are enzymes present in hepatocytes in large amounts. They have been used as indicators of the liver damage by leaking from cells when hepatocytes are damaged and increased in the blood (El-Naggar et al. 2019). Plasma ALT activity was increased in all diabetic groups compared to that in the normal control group. These results were consistent with Barberà et al. (1994) who reported that the activity of ALT increased when diabetes was induced. There was no significant difference in ALT activity among *T.m.* administration groups. However, *T.m.*-1 tended to have the lowest level of ALT activity. Diabetes-induced groups had significantly higher plasma AST activity than the control group, with the *T.m.* administered group showing significantly lower AST activity than the diabetes control group. In a study by Salau et al. (2022) have reported that as a cause of the increase in ALT activity, STZ inactivates the -SH group site, which is the main action point of ALT enzyme function. That has been reported to increase ALT activity in the blood. As a result of this study, increased ALT and AST levels could indicate that complications might occur in the liver caused by diabetes (Rho et al. 1998). Treatment of diabetic rats with *T.m.* reduced activities of these enzymes in plasma samples compared to those in the DC group. Thus, *T.m.* could alleviate liver damage caused by STZ-induced diabetes.

3. Effects of *T.m.* ethanol extract on blood glucose levels

Blood glucose levels were measured at intervals of 3 days to

Table 4. Hematocrit levels and aminotransferase activities of normal and diabetic rats fed extract of *Taraxacum mongolicum*[1)]

Group[2)]	Hematocrit (%)	ALT (KA unit/L)	AST (KA unit/L)
NC	43.8±1.5[NS3)]	14.7± 3.9[a4)]	88.7±3.7[a]
DC	44.8±1.8	78.8±11.9[b]	264.7±58.6[c]
T.m.-1	44.8±2.1	62.7±21.4[b]	188.1±101.9[b]
T.m.-2	45.2±1.7	81.1±21.8[b]	181.5±60.9[b]
T.m.-3	44.2±4.6	70.9±21.2[b]	152.0±45.4[ab]

1) Values are mean±S.D. of 6 rats.
2) See the legend of Table 2.
3) NS: not significant at $p<0.05$.
4) Values with different superscript within the same column are significantly different at $p<0.05$ by Duncan's multiple range test.

figure out effects of *T.m.* on blood glucose level as shown in Table 5. All diabetes-induced groups showed a significant increase ($p<0.05$). Hyperglycemia in diabetic rat is known to be caused by carbohydrate and other metabolic abnormalities. STZ could impair rapid insulin secretion to glucose in pancreatic β-cells. During 14 days of the experiment, all rats in diabetes-inducing groups showed significantly higher plasma glucose levels than the normal control group. After 14 days of administration, concentration-dependent blood glucose lowering effect was shown in *T.m.*-1 and *T.m.*-2 groups compared to a diabetes control group. It was found that the intake of *T.m.* could suppress symptoms of high blood glucose in diabetes. It can explain that the hypoglycemic effect of *T.m.* is likely to increase insulin secretion by further stimulating β-cells (Taniguchi et al. 2006). The hypoglycemic effect of *T.m.* could also be caused by regeneration of pancreatic cells and the presence of insulin-like substances in plants (Arora et al. 2021). These results suggest that *T.m.* could help improve diabetes by reducing increased blood glucose level in STZ-induced diabetic rats.

4. Effects of *T.m.* ethanol extract on lipid profiles

Plasma TC levels are shown in Table 6. The increase in total cholesterol content in diabetes-inducing group might be due to the fact that FFAs, not carbohydrates, are used as energy sources to synthesize lots of cholesterol (Kim et al. 2008a). Diabetes could also be attributed to delayed LDL removal due to reduced LDL receptors, increased production of glycated LDL, which was induced by a lack of insulin, and increased synthesis of very low density lipoprotein (VLDL) in the liver (He et al. 2019; Khalid et al. 2022). According to Ajebli & Eddouks (2019), when diabetes is not controlled, the activity of hydroxymethyl glutaryl-CoA (HMG-CoA) reduction in the liver decreases whereas the activity of HMG-CoA reduction in the intestine increases, resulting in an increase in cholesterol in circulating blood. In the case of the *T.m.*-2 group, from day 1 to day 14 of the experiment, due to relatively low blood glucose levels, plasma TC was significantly reduced compared to that in the diabetes control group.

Plasma TG levels in the diabetic control group were significantly higher than those in the normal control group (Table 7). However, the group with *T.m.* administration did not show any significant difference in plasma TG level from the normal group. The increase in plasma TG in diabetic rats suggests that abnormalities in carbohydrate metabolism could induce lipid metabolism disorder. It has been reported that VLDL production is increased whereas VLDL and chylomicron catabolism is decreased due to decreased LPL activities in peripheral tissues (Siegel et al. 1996). A TG transfer kinetics study (Nikkilä & Kekki 1973) has reported that in diabetes, the rate of conversion of lipid in plasma to TG is increased, which can increase blood TG levels. In the present study, plasma TG levels in groups administered with *T.m.* were significantly lower than those of the diabetic control group, plasma TG of *T.m.* administered group even decreased to control levels. These results suggest that *T.m.* could improve glucose metabolism in diabetic rats and result in decreased TG synthesis in the liver, thereby suppressing the rise of plasma TG (Kim et al. 2008b).

The HDL-chol content of plasma did not show any significant difference between the normal group and the diabetes control group (Table 7). These results were consistent with findings of Durrington & Stephens (1980). They reported that there was no significant difference in plasma HDL-chol content between a diabetes control group and a normal group induced by STZ. However, the *T.m.* treatment group had significantly higher

Table 5. Plasma glucose levels of normal and diabetic rats fed extract of *Taraxacum mongolicum*[1)] (mg/dL)

Group[2)]	0 day	4 day	7 day	11 day	14 day
NC	174.6±8.5$^{a3)}$	152.9±10.9^{a}	150.2±9.4^{a}	148.2±15.1^{a}	175.1±6.6^{a}
DC	527.3±113.3^{b}	802.7±172.7^{b}	749.4±119.5^{bc}	730.3±125.7^{b}	745.9±48.1^{c}
T.m.-1	547.2±42.1^{b}	809.0±138.7^{b}	726.0±100.9^{bc}	746.7±105.1^{b}	557.6±206.4^{b}
T.m.-2	500.8±116.0^{b}	792.4±143.8^{b}	800.3±119.8^{c}	691.8±195.7^{b}	549.2±188.7^{b}
T.m.-3	527.3±78.6^{b}	759.8±68.1^{b}	657.8±144.1^{b}	726.3±176.9^{b}	673.1±166.6^{bc}

1) Values are mean±S.D. of 6 rats.
2) See the legend of Table 2.
3) Values with different superscript within the same column are significantly different at $p<0.05$ by Duncan's multiple range test.

Table 6. Plasma cholesterol levels of normal and diabetic rats fed extract of *Taraxacum mongolicum*[1)] (mg/dL)

Group[2)]	0 day	4 day	7 day	11 day	14 day
NC	76.0±11.4[NS3)]	97.8±19.8[a4)]	86.2±8.0[a]	60.7±7.8[a]	76.2±6.7[a]
DC	78.5±12.4	128.8±23.7[b]	136.4±16.3[b]	106.3±18.5[b]	157.0±20.4[c]
T.m.-1	70.4±11.3	127.0±15.9[b]	133.4±20.1[b]	100.6±20.7[b]	136.5±21.8[bc]
T.m.-2	70.5±5.5	147.6±11.3[bc]	121.2±14.2[b]	88.0±20.0[b]	114.7±21.4[b]
T.m.-3	73.5±6.9	153.6±10.9[c]	136.1±11.0[b]	95.7±10.3[b]	136.9±15.2[bc]

1) Values are mean±S.D. of 6 rats.
2) See the legend of Table 2.
3) NS: not significant at $p<0.05$.
4) Values with different superscript within the same column are significantly different at $p<0.05$ by Duncan's multiple range test.

Table 7. Levels of triglyceride (TG), HDL-cholesterol (HDL-chol) and free fatty acid (FFA) in plasma of normal and diabetic rats fed extract of *Taraxacum mongolicum*[1)]

Group[2)]	TG (mg/dL)	HDL-chol (mg/dL)	FFA (μEq/L)
NC	110.4±8.7[a3)]	36.1±2.1[a]	355.3±51.5[a]
DC	176.9±37.8[b]	48.7±8.0[a]	926.9±103.9[c]
T.m.-1	99.0±38.8[a]	63.8±18.9[b]	726.0±122.2[b]
T.m.-2	115.9±30.7[a]	68.4±3.2[b]	647.2±121.0[b]
T.m.-3	145.8±88.8[ab]	68.4±15.9[b]	693.1±229.0[b]

1) Values are mean±S.D. of 6 rats.
2) See the legend of Table 2.
3) Values with different superscript within the same column are significantly different at $p<0.05$ by Duncan's multiple range test.

HDL-chol levels than the control group. It has been reported that diabetic rats show decreased LPL activity, reduced lipoprotein decomposition, and inhibition of HDL-chol production (Betteridge DJ 2001). In the present study, HDL-chol levels was significantly increased in the *T.m.* treatment group compared to that in the control group. Therefore, *T.m.* administration can increase HDL-chol levels despite diabetes induction.

Plasma FFA content was significantly lower in *T.m.* administrated group than that in the diabetes control group (Table 7). It has been reported that cells can obtain energy sources from fat due to diabetes, which can prevent reesterification of FFAs, resulting in an increase of FFAs in the blood (Karigidi et al. 2020). It has also been reported that hormone-sensitive lipase is activated due to insufficient insulin secretion, which can increase FFAs from stored fat (Tella et al. 2019).

Conclusion

Administration of *T.m.* (2g/kg b.w.) significantly increased body weights of diabetic rats. *T.m.*-1 and *T.m.*-2 groups showed significantly decreased blood glucose and AST than the DC group. In this paper, we found that *T.m.* treatment could decrease plasma glucose and TC, TG, and FFA concentrations of STZ-induced diabetic rats. Therefore, *T.m.* is a good candidate for treating diabetes. Further studies using animal models and human volunteers are needed to develop candidate functional foods based on hypoglycemic activities of *Taraxacum mongolicum*.

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