

Hypoglycemic and Hypolipidemic Effects of Herba Agrimoniae ethanol-extract in T2DM Rats and Data Analysis of Its Dose-Response Relationship

Changlei Li¹, Xiaoqing Chen¹, Baomiao Ma^{2*}

¹School of Medicine, Jiangnan University, Wuhan 430056, China

²Wuhan Institutes of Biomedical Sciences, Jiangnan University, Wuhan 430056, China

Abstract: To observe hypoglycemic and hypolipidemic effects of Herba Agrimoniae ethanol-extract in T2DM Rats and data analysis of its dose-response relationship. Except 10 SPF SD rats were as the control group, the rest 60 T2DM Rats were induced by feeding with high-fat and high-sugar diet and intraperitoneal injection of low-dose streptozotocin (STZ, 35mg/kg). According to the values of blood glucose concentration (≥ 11.1 mmol/L), the rats were randomly divided into diabetic model group, three Herba Agrimoniae ethanol-extract groups of high dose (40g•kg⁻¹), medium dose (20g•kg⁻¹) and low dose (10g•kg⁻¹), and positive control group (glibenclamide, 0.30mg/kg). During 36 d of continuous intragastric (ig) administration, body weights of the rats were measured weekly, the values of fasting blood glucose (FBG) concentration were measured on 7, 14, 21, 28, 35 d after administration. After the last administration, fasting 10h, then, the rats were decapitated after anesthesia. Blood of the rats were collected for the analysis of serum insulin (INS), total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL-C) levels. Compared with the control group, FBG of model group rats were significantly increased ($P<0.01$). After administration 21d, compared with the model group, FBG of Herba Agrimoniae ethanol-extract groups of high dose and medium dose were significantly lower ($P<0.01$ or 0.05); after administration 28d, compared with the model group, FBG of Herba Agrimoniae ethanol-extract groups of high dose, medium dose, low dose and positive control group were significantly lower ($P<0.01$ or 0.05). Compared with the control group, INS levels of model group were significantly lower ($P<0.01$); compared with the model group, INS levels of Herba Agrimoniae ethanol-extract medium dose group were significantly increased ($P<0.05$). Compared with the control group, TC, TG and LDL-C levels of model group rats were significantly increased ($P<0.01$), TG of positive control group was significantly increased ($P<0.05$). Compared with the model group, TC and LDL-C levels of Herba Agrimoniae ethanol-extract medium dose group were significantly lower ($P<0.05$) and TG levels was significantly lower ($P<0.01$); TG levels of positive control group was significantly lower ($P<0.01$). Herba Agrimoniae ethanol-extract can decrease blood glucose, regulate blood lipids in T2DM Rats. Among them, the medium dose group had the best effect.

Keywords: Herba Agrimoniae ethanol-extract; Type 2 diabetics; Blood glucose, Blood lipid; Dose-response relationship

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*Corresponding Author: Baomiao Ma

1. Introduction

Herba Agrimoniae as the whole grass of the perennial herb of rosaceae, which is the ground part of the rosaceae. Which mainly grows in Zhejiang, Jiangsu Hubei and so on. Taste bitter, astringent, temperament. Attributed to the lung, liver and spleen. Modern pharmacological studies have shown that Herba Agrimoniae have such pharmacological activities as hypoglycemic, antitumor, anti-inflammatory, antibacterial, antiviral and insect repellent, and so on, which be used in the clinical treatment of hemoptysis, hematemesis, nosebleed, hemochezia, internal lesion caused by overexertion and such[1,2]. The chemical composition of Herba Agrimoniae are diverse, including flavonoids and glycosides, triphenol derivatives, triterpenoids and glycosides. It has the function of hypoglycemia, anti-tumor, anti-oxidation, anti-inflammation and analgesia, the inhibition of acetylcholinesterase and anti-kinematic fatigue[3,4]. It has the function of hypoglycemic, anti-tumor, anti-oxidation, cardiovascular disease, anti-inflammatory analgesic, inhibition of acetylcholinesterase, anti-exercise fatigue and so on. Researchers found that Herba agrimoniae

ethanol-extract can decrease blood glucose for alloxan-induced diabetic mice, and also showed that significant hypoglycemic effect for diabetic model mice whom caused by glucose, adrenaline and streptozotocin[5,6], but some reports about effects of Herba Agrimoniae ethanol-extract in type 2 diabetic animals not yet found. In this study, through observed some changes for weight, FBG, INS, TC, TG and LDL-C content of T2DM Rats whom be used Herba Agrimoniae ethanol-extract, discussion the pharmacological effects about Herba Agrimoniae ethanol-extract in T2DM Rats.

2. Materials and methods

2.1. Animals

A total of 70 SPF SD rats, half of each are male and female, and their body weight were 200 ± 20 g, whom were conventional breed in barrier environment, eating and drinking with free state. Water and feed were irradiation and sterilized by 60 Co, 12 h/12 h dark cycle.

2.2. Reagents and instruments

Take Agrimony 300g, set 5000 mL round bottom

flask, add 90% ethanol 3000mL reflux extraction 2 h, suction filtration, the residue plus 90% ethanol 2 000mL reflux extraction 2 times each 1h , The filtrate was filtered, the filtrate was combined, ethanol was recovered, concentrated to a concentration of 1 g crude drug/ mL, add an equal volume of pure water washed a total of three times to ethanol-free, dried in a vacuum oven and suspended at 400 mL water extract Herba agrimoniae which be alternated. Streptozotocin, meter, glucose, glyburide, insulin radioimmunoassay kit, total cholesterol assay kit, glycated serum protein assay kit, triglyceride detection kit, low density lipoprotein test kit and so on.

2.3. Modeling and packet administration

Rats be randomized after suitability feeding for a week, control group rats were 10 whom be fed normal diet, and to be made in the model group rats were 60 whom be fed high-fat and high-sugar diet. After the rats were fed four weeks, intraperitoneal injection the STZ for 35mg/kg which be used to make the model. STZ contains citric acid and sodium citrate buffer solution (PH4.4) and the ratio was 1:1.32[7]. Rats were not prohibit drinking but fasted for 12 h before intraperitoneal injection the STZ, the values of FBG concentration were measured on 96 h after administration. The success criteria of in T2DM rats is the blood glucose level ≥ 11.1 mmol/L[8, 9]. After testing, a total of 54 rats were produced model with successfully, the blood sugar of control group rats were normal.

After the models were made with successfully, according to the values of FBG concentration, the rats were randomly divided into diabetic model group, three Herba Agrimoniae ethanol-extract groups of high dose, medium dose and low dose, and positive control group (glibenclamide), the number of each group is 10.

Rats in each group be continued to give high-fat and high-sugar diet for two weeks in order to consolidate the model. Initiation of administration after the model was made with successfully, control group and diabetic model group be given saline with 20ml•kg-1, positive control group be given glibenclamide with 0.30 mg•kg-1, three Herba Agrimoniae ethanol-extract groups of high dose, medium dose and low dose be given ethanol-extract with 40g•kg-1, 20g/kg, 10g/kg respectively , intragastric administration once per day and continued for 36 d, during this time, the rats of each group no death. After the last administration, fasting 10h, the rats were decapitated after anesthesia with 10% chloral hydrate in 3ml/kg and collected blood, the blood were put into the centrifuge with 3500 revolutions per minute and work for 10 m, erum of the rats were collected for the detection and analysis.

2.4. Detection indicators

During 36 d of continuous administration, body weights of the rats were measured weekly. The values of FBG concentration were measured by using the Sino blood glucose meter on 7, 14, 21, 28, 35 d after administration. Follow the instructions to detect the INS content. According to the enzyme assay to detect the TG and TC of serum. By using SUR method to detect the LDL-C.

2.5. Statistical Analysis

Using SPSS 13.0 software for statistical analysis of the obtained data, the results of the indicators were expressed by $\bar{x} \pm s$, significance test using the t test and ANOVA. With $P < 0.05$ was considered statistically significant and $P < 0.01$ as statistically significant difference.

Table 1. Body weight effects of Herba Agrimoniae ethanol-extract in T2DM rats ($\bar{x} \pm s$)

Groups	n	Dose (g/kg)	Weight /g				
			1 st wk	2 nd wk	3 rd wk	4 th wk	5 th wk
Control group	10	—	403±15	415±20	418±26	425±25	420±31
Model group	10	—	307±30 ^a	302±31 ^a	277±23 ^b	263±25 ^b	240±18 ^b
Herba Agrimoniae ethanol-extract group	10	10	306±23	303±19	318±25	311±26	309±22
	10	20	342±25 ^b	353±29 ^b	369±31 ^d	374±30 ^d	398±26 ^d
Positive control group	10	40	325±22	314±23	316±30	308±27	311±25
	10	0.30×10 ⁻³	316±20	317±21	320±25	331±24	315±26

Note: compared with the control group, ^a $P < 0.05$, ^b $P < 0.01$; compared with the model group, ^c $P < 0.05$, ^d $P < 0.01$

3. Results

Body weight effects of Herba Agrimoniae

ethanol-extract in T2DM Rats. with the control group, body weight of model group rats were significantly lower ($P < 0.05$ or < 0.01). Body weight gradually

increase of rats with Herba Agrimoniae ethanol-extract of mediumdose group, compared with the control group, body weight of rats significantly lincrase at 1st and 2nd week (P<0.05), beginning from 3rd week,

body weight gain slowed down, close to blank group (P >0 .05); compared with the model group, body weight of rats significantly increased (P<0.01) from 3rd week. Shown in table 1.

Table 2. FBG and INS effects of Herba Agrimoniae ethanol-extract in T2DM rats (x±s)

Groups	n	Dose (g/kg)	FBG/mmol L ⁻¹					INS (mU L ⁻¹)
			7 th day	14 th day	21 st day	28 th day	35 th day	
Control group	10	—	4.34±1.4	5.32±1.2	6.39±1.5	7.09±1.3	6.88±1.3	357.4±120.3
Model group	10	—	11.23±2.41 ^b	20.14±3.09 ^b	18.69±2.52 ^b	19.13±2.23 ^b	18.76±2.34 ^b	114.9±20.8 ^b
Herba Agrimoniae ethanol-extract group	10	10	10.99±3.03	18.34±2.88	16.49±3.12	15.26±3.38	13.03±3.30 ^{b,c}	126.1±20.4
	10	20	9.66±3.11	15.29±3.67	14.59±4.03 ^c	12.64±3.79 ^c	12.25±2.88 ^{b,d}	157.9±38.2 ^{b,c}
	10	40	10.47±4.09	13.78±4.19	13.09±3.89 ^c	10.59±4.60 ^d	10.11±3.78 ^{b,d}	152.9±67.6
Positive control group	10	0.30×10 ⁻³	11.09±3.38	17.48±3.27	13.26±3.07	11.59±3.67	10.77±3.45 ^{b,d}	129.6±28.7

3.1. FBG effects of Herba Agrimoniae ethanol-extract in T2DM rats

Compared with the control group, FBG of model group rats were significantly increased (P<0.01). After administration 21d, compared with the model group, FBG of Herba Agrimoniae ethanol-extract groups of high dose and medium dose were significantly lower(P<0.01or 0.05); after administration 28 d, compared with the model group, FBG of Herba Agrimoniae ethanol-extract groups of high dose, medium dose, low dose and positive control group were significantly lower (P<0.01 or 0.05). Shown in table 2.

3.2. INS effects of Herba Agrimoniae ethanol-extract in T2DM rats

Compared with the control group, INS levels of

model group were significantly lower(P<0.01). Compared with the model group, INS levels of Herba Agrimoniae ethanol-extract medium dose group were significantly increased (P<0.05). Shown in table 2.

3.3. TC, TG and LDL-C effects of Herba Agrimoniae ethanol-extract in T2DM rats

Compared with the control group, TC, TG and LDL-C levels of model group rats were significantly increased (P<0.01), TG of positive control group was significantly increased(P<0.05). Compared with the model group, TC and LDL-C levels of Herba Agrimoniae ethanol-extract medium dose group were significantly lower (P<0.05) and TG levels was significantly lower (P<0.01); TG levels of positive control group was significantly lower (P<0.01). Shown in table 3.

Table 3. TC, TG and LDL-C effects of Herba Agrimoniae ethanol-extract in T2DM rats (x±s)

Groups	n	Dose (g kg)	TC/mmol L ⁻¹	TG/mmol L ⁻¹	LDL-C/mmol L ⁻¹
Control group	10	—	1.49±0.12	0.67±0.13	0.65±0.11
Model group	10	—	2.14±0.09 ^b	1.58±0.41 ^b	1.05±0.15 ^b
Herba Agrimoniae ethanol-extract group	10	10	2.03±0.45	1.14±0.28	1.01±0.26
	10	20	1.68±0.27 ^c	0.88±0.41 ^d	0.84±0.15 ^c
Positive control group	10	40	2.06±0.45	1.12±0.44	1.01±0.18
	10	0.30×10 ⁻³	1.02±0.18	1.05±0.32 ^{a,d}	1.03±0.22

4. Discussion

Type 2 diabetic is a metabolic disorders which main feature are elevated blood glucose levels and insulin resistance. Now researchers realized that the pathogenesis are insulin resistance and insulin secretion less which lead to the blood sugar and blood lipid concentration at a high level[10,11]. So, the main efficacy of drugs to treatment of type 2 diabetic were decrease blood glucose and regulate blood lipids[12].

Polysaccharide MD 1 component of Herba Agrimoniae have strong efficacy to scavenge radical and inhibit erythrocyte membrane lipid peroxidation, which have some effects for anti-aging, anti-ulcer and anti-inflammatory, anti-cancer, decrease blood glucose, regulate blood lipids and other pharmacological effects[13].

The effective part of the glycemic effect is the ethanol extraction site. On the one hand, Herba agrimoniae can promote the secretion of insulin, thus reduce blood sugar, its mechanism may be through promoting islet cell regeneration, repair and protect the function of islet cells, decrease the blood glucose effect [14]. On the other hand, Herba agrimoniae can improve state of insulin resistance in T2DM rats from aspects of sugar and lipid anti-diabetic effect, its effect mechanism may be anti-inflammatory [15].

The experimental results showed that compared with the model group, body weight of Herba Agrimoniae ethanol-extract of medium dose group rats significantly increased ($P < 0.05$); after administration 28 d, FBG of Herba Agrimoniae ethanol-extract groups of high dose, medium dose and low dose were significantly lower ($P < 0.01$ or 0.05); INS levels of Herba Agrimoniae ethanol-extract medium dose group were significantly increased ($P < 0.05$); TC and LDL-C levels of Herba Agrimoniae ethanol-extract medium dose group were significantly lower ($P < 0.05$) and TG levels was significantly lower ($P < 0.01$).

5. Conclusion

These results indicated that Herba Agrimoniae ethanol-extract can effectively promote insulin secretion, improve insulin resistance and decrease blood glucose, regulate blood lipids in type 2 diabetic, it also open ideas to development new hypoglycemic drugs. About its hypoglycemic mechanism and active ingredients to be further research and exploration.

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