Saudi Journal of Medical and Pharmaceutical Sciences **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

Hypoglycemic and Hypolipidemic Effects of *Hoya parasitica* Variegata (Apocynaceae) and *Crotalaria pallida* Aiton (Fabaceae) Leaves in Aloxane-Induced Type 2 Diabetic Rats

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Original Research Article

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Article History
Received: 13.03.2018
Accepted: 27.03.2018
Published: 30.04.2018

DOI:
10.21276/sjmps.2018.4.4.7

Abstract: Traditional plant treatment for diabetes has produced a rising interest now a day. Therefore, the purpose of this study was to determine the hypoglycemic effect and hypolipidemic properties of the methanolic extract of *Hoya parasitica* Variegata (Apocynaceae) and ethanolic extract of *Crotalaria pallida* Aiton (Fabaceae) in alloxan induced diabetic rats. In this study diabetes was induced in rats by intraperitoneal administration of 100 mg/kg of alloxan. Animals were only treated for one week with both the plant extracts at doses of 300 and 600 mg/kg. Metformin (100 mg/kg) was used as standard for comparison. The antidiabetic effect was examined by measuring blood glucose (BG) level at 0, 3, 5 and 7 days after alloxan treatment. Blood samples were collected after 8 days treatment and analyzed for triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) at sacrifice. Both the plants *H. parasitica* and *C. pallida* at 300 mg/kg and 600 mg/kg showed significant ($p<0.05$) hypoglycemic effect compared to control and untreated diabetic control rats. Alloxan induced diabetic rats showed moderate to significant increases in the levels of BG, TG, TC, LDL-C while body weight, HDL-C and relative weights of liver and pancreas were decreased to controls (non diabetic rats). Administration of both the plant extracts to alloxan induced diabetic rats resulted in significant decrease in BG, TG, TC and LDL-C and the dose 600 mg/kg of both plant extracts were the most effective. HDL-C levels for both plants were markedly increased after treatment compare to untreated diabetic rats. Results of the treatment study showed that both *H. parasitica* and *C. pallida* extracts causes antidiabetogenic properties and beneficial effects on diabetic hyperlipidemia.

Keywords: *Hoya parasitica*, *Crotalaria pallida*, metformin, alloxan, hypoglycemic, hypolipidemic, rat.

INTRODUCTION

Type 2 diabetes is characterized by tissue resistance to the action of insulin combined with a relatively deficiency in insulin secretion. It is also caused by β-cell deficiency which leads to increased triglyceride level and reciprocally low levels of high-density lipoprotein (HDL). Many medicinal plants are used traditionally to treat diabetes mellitus by tribal people. But there is no evidence for the use of these plants. Evidence for the importance of plants in the treatment of type 2 diabetes is necessary. Medicinal plants are less toxic and free from side effect than synthetic ones.

Crotalaria pallida (family: Fabaceae) also known as Kudug Jhunjhuni (Chakma) are extensively used traditionally treat urinary problems, painful swelling of joints, as a vermifuge and to reduce fever. *C. pallida* is reported to have antimicrobial, anti-inflammatory, antioxidant activity [1]. The leaf extract of the plant possessed anti-nociceptive and anti-inflammatory activity [2].This plant is also investigated for its phenol content, actylcholinesterase, xanthine oxidase, lipoxygenase, iron reducing antioxidant, DPPH free radical scavenging properties [3]. Phenol, alkaloids [4], terpenoids [5], saponins [6], steroids and tannins [3], unsaturated fatty acids linolenic and palmitic acid [7], flavonoids and pterocarpanoid, extrafloral nectarines [8], lectin [9], mucronatine, usaramine, nilgirine, mucronatinine and crotastriatine [10]. Luteolin, vitexin, its O-xyloside and chrysoeriol-7-rutinoside, Apigenin has been isolated from leaves and stem bark [11].

Hoya parasitica (family: Apocynaceae) also known as Chera pata (Rema-Kalenga), Fessya gach (Chakma). *H. parasitica* is applied traditionally to treat pain, fever, Jaundice, urinary tract disorders, diabetes and bronchitis. *H. parasitica* is reported to have moderate antibacterial activity [12] and the growth

inhibitory effects of dihydrocanaric acid against both HeLa and SW480 cells [13]. Flavonoids, reducing sugars, tannins, gums and saponins [12], an androstanoid, hoyasterone, a sesquiterpene, 15-bulnesolic acid, and a phenolic compound,1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropan-2-ol, a triterpene, dihydrocanaric acid [13], triterpenic 3, 4-seco acid 3, 4-secolup-20[29]-en-3-oic acid, along with lupeol and lupenone from stem [14] were isolated from *H. parasitica.*

The aim of the study was to experimentally investigate the anti-diabetic and anti-lipidemic effects of *H. parasitica* and *C. pallida* alcoholic extracts used in normal and alloxane-induced Wister rats and to compare it with metformin as a reference standard.

METHODS

Collection of plant material and extraction

The leaves of *H. parasitica* and *C. pallida* were collected from Sylhet hill track in Bangladesh. The plants were identified at Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh where voucher specimen No. number 42022 and 42023 for *H. parasitica* and *C. pallida* respectively have been deposited. The leaves were shade dried and ground into coarse powder.

Methanolic and ethanolic extract

Ground leaves (350g) of *H. parasitica* were soaked in methanol (1.5 L) and ground leaves (350 g) of *C. pallida* were soaked in ethanol (1.5 L) for 7 days. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper. After solvent evaporation under reduced pressure, it rendered a gummy or semisolid concentrates and were designated as crude extracts.

Animals

Adult male and female Wister rats (160-220g) were used in this study and were in animal cases under standard environmental conditions (22-25°C, humidity 40-60%, 12 h light: 12 h dark cycle). Food and tap water were available *ad libitum*. The experiments were conducted in an isolated place and noiseless condition was ensured. All procedures were performed according to our institutional guidelines for animal experimentation.

Induction of diabetes

Animals were allowed to fast for 12 hours. Then diabetes was induced by intra-peritoneal injection of freshly prepared alloxan solution (100mg/kg/BW) in saline water. To overcome drug induced hypoglycemia 10% glucose solution was given. After 3 days blood glucose content was measured by using glucose monitoring system (Match 2, originated from chaina). The rats with blood glucose ≥ 7 mmol/ L were selected for the study. The blood sample was collected from the tail vein of the rats.

Experimental design

In our experiment, a number of 28 rats (24 diabetic rats + 4 normal rats) were used. Diabetes was induced in rats before 3 days of starting the treatment. Albino rats were randomly assigned into group I, II, III, IV, V, VI, VII ; 4 rats in each group for the respective one week treatment for the determination of blood glucose, lipid profile test studies.

- Group I: Normal Control (Non Diabetic)
- Group II: Diabetic Control (Untreated Group)
- Group III: Diabetic Control + Metformin (100 mg/Kg)
- Group IV : Diabetic Control + Methanolic extract of *H. parasitica* (300 mg/kg)
- Group V: Diabetic Control + Methanolic extract of *H. parasitica*(600 mg/Kg.)
- Group VI: Diabetic Control + Ethanolic extract of *C. pallida* (300 mg/Kg.)
- Group VII: Diabetic Control + Ethanolic extract of *C. pallida* (600 mg/Kg)

All test drugs and extracts were administered by orally for one week. Body weight and relative organ weight of liver and pancreas also recorded. After one week treatment, all rats were killed by cervical dislocation. Blood sample were collected from rats to get the serum. The concentration of TC, TG, LDL and HDL Cholesterol were measured calorimetrically by blood analyzer using commercially available wet reagent diagnostic kits (HUMAN GmbH, Germany).

STATISTICAL ANALYSIS

Data are expressed in mean ± SEM. Statistical analysis was carried out in one-way ANOVA followed by post-hoc Dunnet test. Comparisons with p values <0.05 were considered to be statistically significant.

RESULTS

Effect of *H. parasitica* on blood glucose level

Table 1 shows the effects of methanolic extracts of *H. parasitica* leaves on blood glucose levels of Wister rats after one week of continuous treatment. Sequential injections of alloxan caused a significant increase in blood glucose concentrations in all groups of rats compared with their respective baseline blood glucose (at the time of grouping) and to control values. At all-time points, blood glucose concentrations remained unchanged in normal rats treated with distilled water. However, oral administration of the plant extracts (300 & 600 mg/kg) as well as metformin (100 mg/kg) to diabetic rats decreased significantly ($p<0.05$) in blood glucose concentrations. The blood glucose level decreased in case of metformin (63.09%) and *H. parasitica* extract of 300 and 600 mg/kg (56.49% and 45.88%) respectively compared to untreated diabetic group rats.

Effect of *C. pallida* on blood glucose level

The effects of ethanolic extract of *C. pallida* leaves and metformin on blood glucose level in alloxan induced diabetic rats were shown in the table 1 which represented that the blood glucose level significantly decreased ($p<0.05$) after administration of metformin and *C. pallida* extract (300 & 600 mg/kg). The blood glucose level decreased in case of metformin (63.09%) and *C. pallida* extract 300 and 600 mg/kg (50.85% and 52.74%) compared to untreated diabetic rats.

Table-1: Effects of *H. parasitica* and *C. pallida* on blood glucose level in alloxan induced type 2 diabetic rats

Groups	Fasting blood glucose level (mmol/l)				
		Day of treatment			
	At the time of grouping	Day 0	Day 3	Day 5	Day 7
Control	4.68 ± 0.23	4.85 ± 0.23	5.03 ±0.26	5 ±0.29	4.9 ± 0.26
Untreated diabetec	5.5 ± 1.04	15.8 ± 0.93***	15.88 ±0.76***	15.53 ±0.58***	15.55 ± 0.71***
Diabetic + Metformin (100mg/kg)	5 ± 0.15	13.28 ± 0.18***	8.55 ±0.39$^{**\#\#\#}$	6.78 ±0.28$^{*\#\#\#}$	4.9 ± 0.27$^{**\#\#\#}$
Diabetic + *H. parasitica* (300mg/kg)	4.98 ± 0.12	14.28 ± 1.25***	11.13 ± 0.98$^{***\#\#\#}$	9.3 ± 1.02$^{***\#\#\#}$	7.73 ± 1.03$^{**\#\#\#}$
Diabetic + *H. parasitica* (600mg/kg)	7.8 ± 0.73	14.65 ± 1.22***	11.3 ± 0.75$^{***\#\#}$	8.35 ±0.42$^{**\#\#\#}$	6.38 ± 0.49$^{\#\#\#}$
Diabetic + *C. pallida* (300mg/kg)	3.93 ± 1.16	14.75 ± 4.51**	3.73 ±0.62$^{***\#\#\#}$	6.18 ±1.54$^{\#\#\#}$	7.25 ± 1.27$^{*\#\#\#}$
Diabetic + *C. pallida* (600mg/kg)	6 ± 0.96	13.7 ± 0.39**	10.63±0.46$^{***\#\#\#}$	8.45 ±0.47$^{*\#\#\#}$	6.48 ± 0.59$^{\#\#\#}$

Values are means ± SEM; number of animals per group = 4.

$^{*;}$ P <0.05; **: P<0.01; ***: P<0.001: significantly different compared to control.

$^{\#}$: p<0.05; $^{\#\#}$: p<0.01; $^{\#\#\#;}$ p<0.05: significantly different compared to untreated diabetic.

Effect of *H. parasitica* on lipid profile

Table 2 showed that the effect of the *H. parasitica* extract on TG, TC, HDL-C and LDL-C in alloxan induced diabetic rats. After seven days treatment with *H. parasitica* extracts (300 & 600 mg/kg) and metformin (100 mg/kg) the result showed a significant decrease in ($p<0.05$) TG, TC, LDL-C levels. The TG level decreased in case of metformin (53.97%) and *H. parasitica* extract of 300 and 600 mg/kg (19.12% and 20.50%) respectively compared to untreated diabetic group rats.

Effect *C. pallida* on lipid profile

This study showed significant decrease ($p<0.05$) in TG, TC, LDL-C levels and while significant increase ($p<0.05$) in HDL-C levels in diabetic rats treated with *C. pallida* extracts (300 & 600 mg/kg) and metformin (100 mg/kg) compared to normal control rats. The administration of *C. pallida* extract of 300 mg/kg decreased TG, TC, LDL-C levels at 20.35%, 19.71%, 46.75% and HDL-C levels increased at 30.34%. The *C. pallida* extract of 600 mg/kg decreased TG, TC, LDL-C levels at 23.11%, 21.08%, 57.46% while HDL-C levels increased at 45.49%. The administration of metformin of 100 mg/kg decreased TG, TC, LDL-C levels at 53.97%, 49.85%, 73.85% and HDL-C levels increased at 67.12%.

Table-1: Effects of *H. parasitica* and *C. pallida* on lipid profile in alloxan induced type 2 diabetic rats

Group	Lipid profile (mmol/l)					
	Initial Body wt.	Final Body wt.	TG	TC	HDL-C	LDL-C
Control	222± 17.05	240.5± 17.05	4.01± 0.13	6.34± 0.68	7.84± 0.43	2.17± 0.95
Untreated diabetic	202.5± 3.21*	195.75± 4.77*	6.90± 0.50*	10.01± 0.67*	5.79± 0.55*	10.69± 1.34
Diabetic + Metformin (100mg/kg)	177± 2.48#	182.5± 3.53#	3.18± 1.18*#	5.02± 0.58*#	9.68± 0.56*#	2.79± 0.45*#
Diabetic + H. parasitica (300mg/kg)	170.25± 1.03#	163.25± 3.57#	5.58± 0.21*#	7.91± 0.57*#	7.84± 0.64*#	5.35± 0.59*#
Diabetic + H. parasitica (600mg/kg)	167.75± 4.84#	168.5± 5.61#	5.48± 0.21*#	7.74± 0.26*#	8.15± 0.25*#	4.88± 0.24*#
Diabetic + C. pallida (300mg/kg)	168± 4.26#	162.25± 3.88#	5.49± 0.27*#	8.04± 0.42*#	7.55± 0.49*#	5.69± 0.67*#
Diabetic + C. pallida (600mg/kg)	162± 4.88#	162± 3.70#	5.31± 0.26*#	7.9± 0.32*#	8.43± 0.74*#	4.55± 1.14#

Values are means ± SEM; number of animals per group = 4.

Values are means ± SEM; number of animals per group = 4.

*: P <0.05: significantly different compared to control.

#: p<0.05: significantly different compared to untreated diabetic.

DISCUSSION

Being an under developed country most of the people of Bangladesh are unable to afford expensive antidiabetic agents and depends on plants as alternatives. In this experiment our studied plants *H. parasitica* and *C. pallida* showed significant antidiabetic as well as antilipidemic effect compared to control and untreated diabetic rats.

Pancreas is the primary organ that maintains homeostasis of glucose in the blood and in response to elevate blood glucose level insulin is secreted (15). Alloxan which has a destruction effect on β-cells of the islets of Langerhans used for the induction of diabetes mellitus (16, 17) that causes an enormous reduction in insulin release thereby inducing hyperglycaemia (18). This condition ultimately leads to various metabolic alterations in the animals like increased blood glucose, increased cholesterol etc [19, 20]. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage.

In the present investigation *H. parasitica* and *C. pallida* leaf extracts confirmed the considerable anti-diabetic activity that confirms the possibility that the major functions of the extracts are on the protection of vital tissues of the pancreas, thereby reducing the causation of diabetes in the experimental animals. The possible mechanism includes the stimulation of β-cells to produce insulin and subsequent release of insulin, activation of the insulin receptors and decreasing absorption of sugar from the intestine.

The results from the present study also signified that the plants extracts can reduce the levels of triglyceride, total cholesterol, low density lipoprotein cholesterol while increase high density lipoprotein cholesterol. The increase in insulin secretion and the consequent decrease in blood glucose level may lead to stimulation of fatty acid biosynthesis (Insulin stimulates lipid synthesizing enzymes (fatty acid synthase, acetyl-CoA carboxylase) and also the incorporation of fatty acids into triglycerides in the liver and adipose tissue). In the presence of insulin, the hormone-sensitive lipase will be inhibited in the adipose tissue, and mobilization of fatty acid from adipose tissue by glucagons will also be inhibited and therefore leading to the observed decrease plasma level of free fatty acids [21].

The result of our study comply with the findings of other reports [22, 23] included many plants extracts with potential therapeutic value in fighting artherosclerosis a major complication of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level [24]. Thus, the best treatment for diabetes should be to control glycemia along with the control of lipid profile. Several studies have shown that an increase in HDL-cholesterol is associated with a decrease in coronary risk and most of the drugs that decrease total cholesterol

also increase HDL-cholesterol [24]. Reduced TG and LDL associated with the increased HDL with the administration of *H. parasitica* and *C. pallida* extracts to diabetic rats is the important finding of this experiment.

CONCLUSION

Our study has shown that the methanlic extract of *H. parasitica* and ethanolic extract of *C. pallida* are very effective in glucose lowering effect in alloxan induced diabetic rats. These extracts exert antidiabetic as well as serum lipid lowering effects in alloxan induced diabetic rats and if the plants extracts used as hypoglycemic agent, may also reverse dyslipidemia associated with diabetes and prevent the cardio vascular complications that are very common in diabetic patients**.** Further studies on this plant will focus on bioassay-guided isolation of the active principles from this plant.

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