

## Effect of Honey Bee Venom (*Apis mellifera*) on Hyperglycemia and Hyperlipidemia in Alloxan Induced Diabetic Rabbits

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### Abstract

**Introduction:** In diabetes therapy, a great attention is paid on lowering blood glucose levels and lipid regulating mechanisms of various medical agents including animal toxins. Honey bee venom reduces blood glucose level through increased insulin secretion and glucose take-up. It also has lipid regulating activity verified in several other studies. For that reason, bee venom could be considered as a potential remedy for diabetes. This study aims to investigate the effect of Mongolian honey bee venom on hyperglycemia and hyperlipidemia in alloxan induced diabetic rabbits.

**Material and method:** Twenty two Chinchilla rabbits were divided into three groups: the control (n=6), the diabetic (n=8), and the bee venom treated (n=8) groups. The diabetic group was injected with a 5% solution of Alloxan monohydrate at 100 mg/kg intravenously via the marginal vein behind the ear for 2 minutes to induce the diabetes. The bee venom treated group received a bee sting (a sting contains 0.2-0.5 ml of bee venom) on their hind paw every other day after the confirmation of diabetes.

**Result:** Bee venom treatment (BVT) led to the following changes: compared to the diabetic group, the bee venom treated group's blood glucose levels decreased by 14.9% -26.5%; blood cholesterol levels reduced by 12.5%-19.1%; Low Density Lipoproteins (LDL) levels lowered by 11.2%-14.2%; and High Density Lipoproteins (HDL) levels increased by 2.5% - 26.25%.

**Conclusion:** Bee venom lowers blood glucose levels and improves lipid profile in alloxan-induced diabetic rabbits and can be considered as a therapeutic agent for diabetes. Further studies should be carried out to determine the most appropriate bee venom dose for the best therapeutic effect.

**Keywords:** Bee Venom Treated (BVT); Rabbit; Diabetes; Alloxan; Glucose; Cholesterol; Triglyceride; LDL; HDL

### Introduction

Diabetes is a metabolic disorder that is characterized by chronic high blood glucose levels that causes complications in the eyes, kidneys, heart, vessels and nerves. Another serious pathogenesis of diabetes is an abnormal lipid profile indicated by low levels of HDL and high concentration of triglyceride and LDL. Hence the potential remedy for diabetes not only needs the blood glucose levels lowering action, but also lipid regulating effect.

Major component of bee venom are melittin and phospholipase A2, a polypeptide and an enzyme that increase insulin secretion from pancreatic  $\beta$ -cells via depolarization of beta cell membrane [1]. Another potential mechanism for bee venom blood glucose and cholesterol reducing action is lipolytic properties of BV. The components partially lyse cell membrane which increases glucose transport and lipid take-up into adipose tissue. Based on the above properties, BV could be considered as a therapeutic agent for diabetes. This study aims to investigate the effect of Mongolia's bee venom on blood glucose, cholesterol, low density lipoprotein and high density lipoprotein levels in diabetic subjects.

### Materials and Method

Twenty two Chinchilla rabbits weighting around 1.7-2.5 kg were obtained from "Biocombinat" animal farm based in Ulaanbaatar, Mongolia. Subjects were divided into three groups: the control

(n=6), the diabetic (n=8), the bee venom treated (n=8) groups. This experiment continued for 14 days.

The first group of healthy rabbits was injected with distilled water 2 ml/ kg every other day. The rabbits form the second group was injected with a 5% solution of Alloxan monohydrate (Sigma Chemical Co., USA), a single dose at 100 mg/kg intravenously via marginal ear vein for 2 minutes to induce diabetes. One to two minutes later a 0.9% NaCl solution 7 ml/kg to body weight was injected intravenously to prevent nephropathy. 27.5% glucose (3.5-4 ml/kg) was administered subcutaneously 4 and 6 hours after injection to offset transient hyperglycemia developing after alloxan treatment [2,3]. Blood glucose level at 15 mmol/l and higher reveals the diabetic condition.

The third group which was treated with bee venom, received a bee sting (a sting contains 0.2-0.5 ml of bee venom) on their hind paw every other day after confirmation of diabetes.

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Blood samples from all groups were collected on days 1,3,7 and 14 in a fasting state from rabbits' marginal ear vein by 26 G needle and syringe [4]. Blood glucose levels, plasma cholesterol levels, triglyceride levels, LDL and HDL levels were determined by "Humylazer 2000" analyzer (Human, Germany).

The values were expressed as mean ± S.E.M, Statistical analyses were performed by SPSS-16 one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test for multiple comparisons. P<0.05 was considered as significant.

## Results

In the diabetic group alloxan administration dramatically increased blood glucose level from  $5.52 \pm 0.18$  mmol/l to  $21.65 \pm 2.2$  mmol/l. Blood glucose level in this group remained 3.9 to 5.5 times higher compared to the control group throughout the experimental days. After inducing the diabetic condition, blood glucose levels in the Bee venom Treated (BVT) group progressively increased to  $15.92 \pm 1.53$  mmol/l on day 1, and reaching its highest value at  $26.38 \pm 3.61$  mmol/l on day 3, and then steadily decreasing to  $25.93 \pm 2.35$  mmol/l and  $22.00 \pm 2.47$  mmol/l on days 7 and 14. Yet compared with the diabetic group, glucose values in the BVT group were lower by 26.5% on day 1, and 13.3% on day 3. On 7<sup>th</sup> and 14<sup>th</sup> days, BVT group's glucose levels maintained 14.9 % and 21.4% lower than the diabetic group (P<0.05) (Figure 1).

Plasma cholesterol level in the diabetic group reached  $4.92 \pm 0.51$  mmol/l on the first day, 72% higher than the control group. Cholesterol level hit the maximum value of  $5.72 \pm 0.85$  mmol/l on day 3 and then steadily decreased until  $4.57 \pm 0.43$  mmol/l by day 14. Bee venom treatment in diabetic rabbits lowered plasma cholesterol levels compared to the diabetic group by 3.5% at  $4.75^* \pm 0.32$  mmol on the 1<sup>st</sup> day. On the following days, cholesterol levels in the BVT group showed significant decrease compared with the diabetic group, between the second and the third groups were 11% on day 3, 8% on day 7, and 10.9 % on day 14 (Figure 2). Cholesterol level in the BVT group was approaching the control group values of  $3.20 \pm 0.15$ , and reached  $4.07 \pm 0.50$  mmol/l on day 14.

The effect of bee venom treatment on plasma triglyceride (TG) content in diabetic rabbits is illustrated in Figure 3. Diabetes causes a significant increase in plasma triglyceride content. Levels of plasma triglyceride in the diabetic group on the day 1 increased to  $2.59 \pm 0.21$  mmol/l, 2.2 times of the control group. For the following experimental days, triglyceride levels in the diabetic group increase to values of  $3.17 \pm 0.08$  mmol/l on day 3,  $2.96 \pm 0.25$  mmol/l on day 7, and  $2.78 \pm 0.42$  on day 14. Compared to the diabetic group, plasma triglyceride in the BVT group was considerably lower during the course of the experiment. Triglyceride levels in the BVT group increased to  $2.24 \pm 0.15$  mmol/l on day 1, yet this value was 12.5% lower compared to the diabetic group. On day 3, plasma triglyceride levels reached its highest value of  $2.73 \pm 0.03$  mmol/l, then gradually declined to  $2.59 \pm 0.04$  mmol/l on day 7, and  $2.25 \pm 0.12$  mmol/l on day 14, remaining from 13.9 % to 19.1% lower than the diabetic group (P<0.05) (Figure 3).

In Figure 4, the diabetic group shows an increase in plasma LDL levels to  $2.97^* \pm 0.32$  mmol/l, 42.1% higher than the control group (P<0.05). On days 3 and 7, LDL levels varied from  $2.90 \pm 0.27$  mmol/l to  $3.05 \pm 0.72$  mmol/l and dropped to  $2.61 \pm 0.53$  mmol/l by day 14.

Plasma LDL levels in the BVT group were  $2.69 \pm 0.42$  mmol/l, 9.4% lower than the diabetic group on day 1. By day 3 LDL levels increased to  $3.05 \pm 0.57$  mmol/l and were 5.2% higher compared to the diabetic group. On days 7, LDL levels in the BVT group sharply decreased to

$2.71 \pm 0.24$  mmol/l and continued dropping down to  $2.24^* \pm 0.28$  mmol/l by day 14, 14.2 % lower than the diabetic group which was very close to the control values (P<0.05).

In Figure 5, plasma HDL levels in the control group decreased to  $1.66 \pm 0.07$  mmol/l on day 1, around 3% - 6.4% lower compared to the control group and stayed stable throughout the experiment.

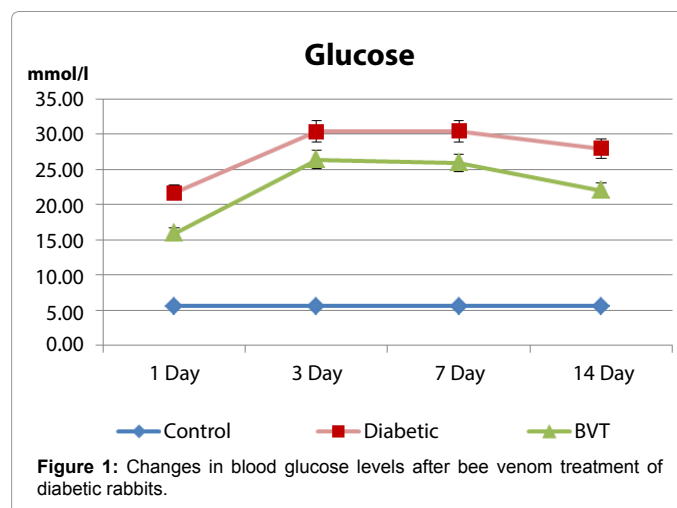


Figure 1: Changes in blood glucose levels after bee venom treatment of diabetic rabbits.

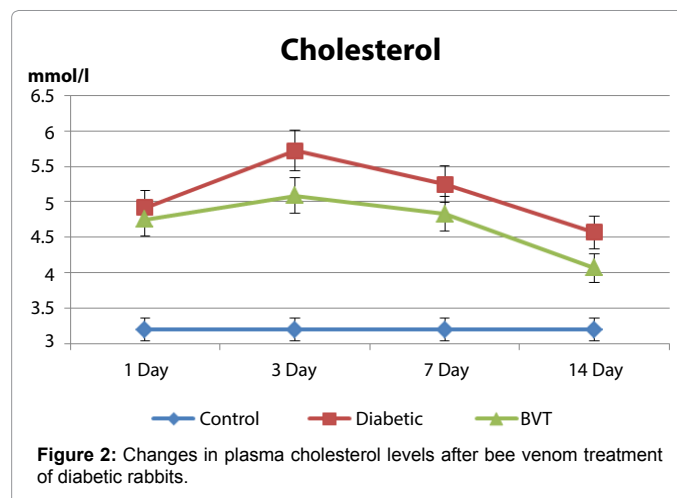


Figure 2: Changes in plasma cholesterol levels after bee venom treatment of diabetic rabbits.

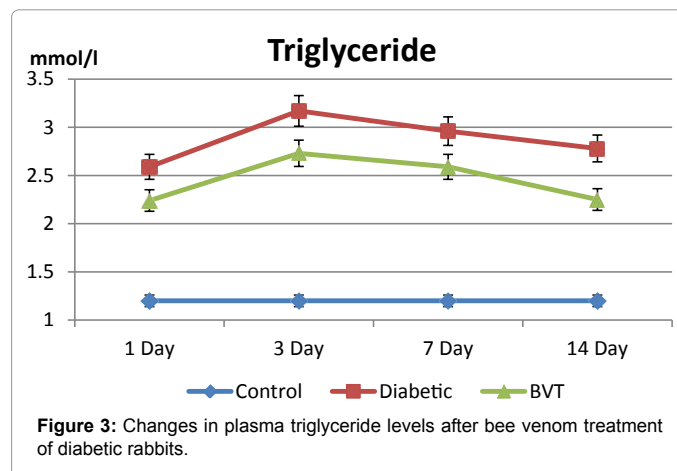
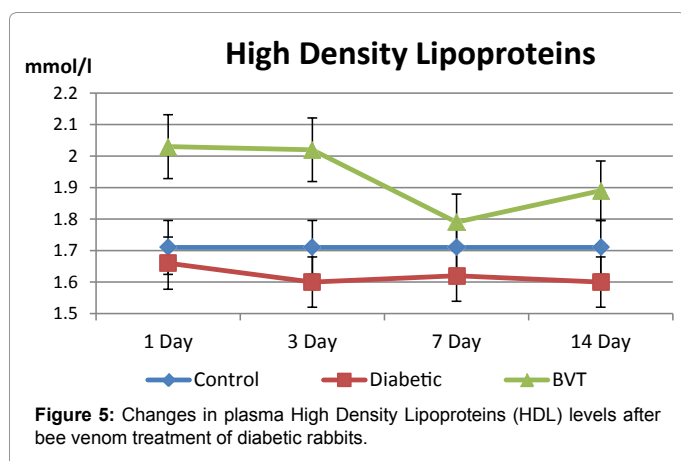
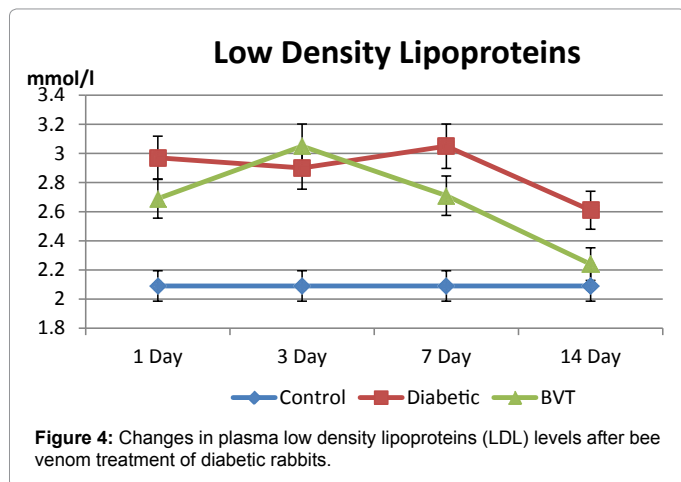


Figure 3: Changes in plasma triglyceride levels after bee venom treatment of diabetic rabbits.



However, bee venom treatment increased plasma HDL levels to  $2.03 \pm 0.33$  mmol/l on day 1, 122% of the diabetic group. The difference between the BVT group and the diabetic groups increased to 26.3% on day 3, 10.5% on day 7, and 18.1% on day 14 (Figure 5). Even compared to the control group, HDL levels after bee venom treatment became

18% higher on day 1 then remained at least 10.5 % higher during the experimental days ( $P < 0.05$ ) (Table 1).

## Discussion

In this study, bee venom treatment showed blood glucose levels lowering activity in alloxan induced diabetic rabbits. Alloxan monohydrate induces type 1 diabetes in experimental rabbits through exclusive destruction of insulin producing beta cells in pancreas [5].

The bee venom treatment lowered plasma glucose, cholesterol, triglyceride, and LDL levels; and increased HDL levels in diabetic rabbits compare to untreated diabetic group. Our results were consistent with findings of Mousavi et al. which also confirmed hypoglycemic and hypolipidemic activity of bee venom in diabetic mice [6]. In another study, bee venom reduces glycaemia and cholesterolemia in healthy subjects depending on the inoculated dose [7]. These effects could be attributed to melittin and phospholipase A2, a polypeptide and an enzyme that altogether make up to 62% of the bee venom. One mechanism for BV to lower blood glucose levels is through the suppression of beta cell inflammation [8] and direct stimulation of insulin secretion [1,9]. Fujimoto et al. studied agonist properties of phospholipase A2 and melittin and discovered that they induce monophasic release of insulin from beta cell [9]. Melittin initiates membrane depolarization which leads to increased inflow of  $Ca^{2+}$  ion to beta cells, through calcium channel depending on the extracellular calcium [10,11].

According to Ginsberg (1996), another possible strategy to treat diabetic dyslipidemia is to link glucose and fatty acid metabolism by improving insulin action in fat cells which result in lower LDL, triglyceride and in increased HDL levels [12]. The bee venom's phospholipase A2 partially lyses cell membrane due to its enzymatic action on the plasmatic lipoproteins [13]. This activity increases glucose transport and lipid take-up into adipose tissue through partial lyses of adipocytes membrane and binding of higher number of insulin molecules [14]. Some studies suggest that bee venom phospholipase A2 has higher affinity to the plasmatic lipoproteins and exerts its cytotoxic effect by generating free fatty acids and lipophospholipids, thus free cholesterol in HDL is esterified [15]. Phospholipase A2 enzymatic action plays the central role in the described mechanism for reducing

Measured biochemical parameters	Groups	Blood sample drawing days			
		Day 1	Day 3	Day 7	Day 4
Glucose mmol/l	Control	$5.52 \pm 0.18$	$5.52 \pm 0.18$	$5.52 \pm 0.18$	$5.52 \pm 0.18$
	Diabetic	$21.65 \pm 2.2$	$30.42 \pm 2.51$	$30.47 \pm 1.95$	$28.00 \pm 1.02$
	BVT	$15.92 \pm 1.53$	$26.38 \pm 3.61$	$25.93 \pm 2.35$	$22.00 \pm 2.47$
Cholesterol mmol/l	Control	$3.20 \pm 0.15$	$3.20 \pm 0.15$	$3.20 \pm 0.15$	$3.20 \pm 0.15$
	Diabetic	$4.92 \pm 0.51$	$5.72^* \pm 0.85$	$5.25 \pm 0.67$	$4.57^{**} \pm 0.43$
	BVT	$4.75^* \pm 0.32$	$5.09^* \pm 0.40$	$4.83 \pm 0.23$	$4.07^* \pm 0.50$
Triglyceride mmol/l	Control	$1.20 \pm 0.07$	$1.20 \pm 0.07$	$1.20 \pm 0.07$	$1.20 \pm 0.07$
	Diabetic	$2.59^{**} \pm 0.21$	$3.17^* \pm 0.08$	$2.96 \pm 0.25$	$2.78 \pm 0.42$
	BVT	$2.24 \pm 0.15$	$2.73^* \pm 0.03$	$2.59 \pm 0.04$	$2.25 \pm 0.12$
LDL mmol/l	Control	$2.09 \pm 0.08$	$2.09 \pm 0.08$	$2.09 \pm 0.08$	$2.09 \pm 0.08$
	Diabetic	$2.97^* \pm 0.32$	$2.90^{**} \pm 0.27$	$3.05^* \pm 0.72$	$2.61 \pm 0.53$
	BVT	$2.69 \pm 0.42$	$3.05 \pm 0.57$	$2.71 \pm 0.24$	$2.24^* \pm 0.28$
HDL mmol/l	Control	$1.71 \pm 0.05$	$1.71 \pm 0.05$	$1.71 \pm 0.05$	$1.71 \pm 0.05$
	Diabetic	$1.66 \pm 0.07$	$1.60 \pm 0.10$	$1.62 \pm 0.09$	$1.60 \pm 0.09$
	BVT	$2.03 \pm 0.33$	$2.02 \pm 0.60$	$1.79 \pm 0.40$	$1.89 \pm 0.28$

BVT- group of diabetic rabbits treated with bee venom; LDL - low density lipoprotein levels in plasma; HDL - high density lipoprotein levels in plasma; The observations are expressed as a mean  $\pm$  S.E.M. \* $p < 0.05$  \*\* $p < 0.01$ , as compared to control group.

**Table 1:** Dynamics of blood glucose levels, plasma cholesterol levels, triglyceride levels, LDL levels and HDL levels in control, diabetic and bee venom treated diabetic rabbits.

cholesterol, triglyceride, LDL and in increasing HDL; and regulating a lipid profile.

The third action mechanism brought up by Kim et al. proves that bee venom has immune-modulating effect which inhibit onset of type 1 diabetes in non-obese diabetic mice, often caused by underlying autoimmune processes that damage pancreatic beta cells [16].

## Conclusion

1. Mongolian bee venom exerts hypoglycemic activity on alloxan-induced diabetic rabbits through suppression of pancreatic beta cell inflammation, promotion of insulin secretion and promotion of glucose uptake in adipose tissue.

2. Mongolian bee venom exerts hypolipidemic activity on alloxan-induced diabetic rabbits due to improvement of lipid uptake into adipose tissue and hydrolysis of triglyceride.

3. Mechanisms of bee venom for lowering blood glucose and plasma lipid in diabetic subjects should be studied further.

Compared to this study, Other researches on bee venom's action on blood glucose levels, plasma cholesterol levels, and triglyceride levels used about 2-12 times higher doses of bee venom. Therefore further studies on the most appropriate dose of bee venom for the best therapeutic effects on the diabetes shall be studied.

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