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Apis cerana Bee Venom: It's Anti-Diabetic and Anti-Dandruff Activity Against *Malassezia furfur*

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Abstract: Bee Venom (BV) is a highly protein rich substance secreted by the worker and queen bees. It contains pharmacologically important constituents. Melittin is the major constituent present in bee venom and it has been reported to have anti-diabetic and anti-microbial property. Application of bee venom has shown its efficiency for various ailments. For the present study two populations namely test and control were choosen for the anti diabetic activity and the test population was categorised as before stinging and after stinging by *Apis cerana* bees. The results were compared with the standard values of the blood sugar, cholesterol and triglycerides. The test populations showed very low blood sugar, cholesterol and triglyceride compared to the control population. In test population the average count of eosinophil was around 7%, normal range is around 1-5%. This increase in eosinophil count is due to release of histamine from the bee venom. The lower levels of blood sugar and cholesterol in test population are evident for the anti diabetic activity of bee venom. *Malassezia furfur,* dandruff causing lipophilic yeast was tested for its susceptibility to bee venom. Ketoconazole was used as reference standard. Out of the five different venom concentrations used, 5mg/ml concentration of *A. cerana* venom showed 86.9 mm^2 inhibition. Thus value added natural products of bee venom can have a great impact in the near future to control diabetes mellitus, dandruff and various other afflictions.

Key words: *Apis cerana* · Bee venom · Anti-diabetic · *Malassezia furfur* · Ketoconazole

INTRODUCTION

Honeybees are a subset of bees in the genus *Apis*, mainly involved in the foraging activity to collect nectar and pollen. There are more than 20,000 species of honeybees, of that five species are considered to be the most important. Out of the five important bee species *A. cerana* is found in Southern and South eastern Asia. Bee Venom (BV) is a highly protein rich substance secreted by the worker and queen bees. It contains pharmacologically important constituents. So far only eighteen have been characterized in *Apis mellifera* venom. The important constituents present in bee venom are Melittin having 50% of dry weight of the bee venom, mast cell degranulating peptide (MCD) 3%, Apamin 3%, Hyaluronidase 3%, Phospholipase 12% and Histamine 0.9%. Melittin is considered to be the major constituent of bee venom.

Application of bee venom has shown its efficiency in various ways. Bee venom (BV) is given as a shot for rheumatoid arthritis and other ailments [1]. It has been reported that venom shows a good anti-diabetic effect by increasing the insulin level in mice models [2]. *Apis mellifera* venom has been tested against various clinical pathogens [3] dermatophytes such as Trichosporon [4] which showed good inhibition against the pathogens. *Malassezia furfur* is a kind of a dermatophyte that infects the scalp and causes irritation. Most of the human population is affected by this infection. It is a lipophilic fungus. Skin infections caused by *M. furfur* are pityriasis versicolor, Malassezia folliculitits and Seborrhoeic dermatitis [5]. These diseases are considered as contagious and may affect any age group [6].

The main objective of the present study is to investigate the antidandruff activity of *Apis cerana* bee venom against *M. furfur* species

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causing dandruff and the effect of bee venom on the blood parameters of human for the anti diabetic activity.

MATERIALS AND METHODS

Venom Collection: *Apis cerana* colonies were obtained from the University of Agricultural Sciences Apiary, Bangalore. The bee venom collector was procured from Chung Jin Biotech Co. Ltd, Korea for collecting the bee venom from *Apis cerana* bees. Bee venom was collected through the venom collector with a mild electric shock given to the honeybees [7]. The venom collector was placed near the entrance of the hive and it was assessed that the steel wires were facing down. The wires were alternately charged to a maximum of 12 volts. When shock was given, the bees were literally irritated and they ejected the venom on the glass plate which was like a white liquid and later dried. The shock given to the honeybees were mild and it did not kill the bees but only irritated them to eject the venom. The venom was later scraped off with a clean sterilized knife/blade and stored under -20°C until use.

Venom Preparation: The collected venom was completely dissolved in autoclaved double distilled water and prepared to concentration of 60mg/ml and maintained as stock. Different concentrations in the range of 1-5mg/ml were prepared from the stock and used for testing the anti-dandruff activity.

Preparation of Test Organism: *Malassezia furfur*, a dandruff causing organism was purchased from MTCC, Chandigarh, (MTCC No: 1374). The culture was obtained as a slant. Saboraud's Dextrose broth (SDB) supplemented with corn oil was used for preparing the culture. Plating was done with the medium of following composition: Dextrose 40g/l, Peptone 30g/l, Agar 18g/l supplemented with corn oil [8]. Culturing was done for 7 days and aerobic condition was maintained. The temperature was maintained at 30±2°C [9].

Anti-Diabetic Activity: For testing the anti-diabetic potential of bee venom, two groups containing five members of human population of same age, weight, sex, food habit and locality were selected. It was confirmed that there were no previous history of immunization in both the test groups. One group was the test population who were beekeepers constantly exposed to bee venom by the bee sting and the other group was the control who were normal human population not being exposed to bee sting. Blood samples were collected from both the groups. In order to compare the results, blood from test population were collected twice i.e. before stinging by the bees and after being stung by bees. Eight *Apis cerana* bees were made to sting the test group individually. The blood samples were collected after thirty minutes of bee stinging from the test population. All the blood samples were tested for the levels of blood sugar, insulin, triglyceride, cholesterol [2] and the total blood count.

Growth Curve Studies of Malassezia Furfur: Growth curve studies were carried out by inoculating the culture to Saboraud's Dextrose broth (SDB) and kept in a shaker at 140rpm and the absorbance was measured from zeroth hour till it attains stationary phase. Absorbance was read at 530nm in UV-Visible spectrophotometer [10]. The results were noted and the graph was plotted. μ_{max} and doubling time were calculated according to the formula.

μ_{max} = $dx/dt\ (1/x)$; 1/ h
dx = $x_2 - x_1$
dt = $t_2 - t_1$; h
x = $(x_1 + x_2)/2$; OD units
Td = $0.693/\mu_{max}$; h

Maximal OD corresponded to OD in stationary phase.

Anti-Dandruff Activity: For testing the susceptibility of *M. furfur* for venom, fully grown culture maintained in SDB was spread onto petriplates with the medium of above mentioned composition. 200mg/ml Ketoconazole was used as reference standard. Disc diffusion was followed for measuring the zone of inhibition [4]. 6mm discs were used throughout the experiment. Five different concentrations, 1, 2, 3, 4 and 5mg/ml concentrations of venom were tested against *M. furfur*. 10µl each of the above mentioned concentration were loaded onto the disc and placed on the plates spread with *M. furfur*. Water was used as control. The level of inhibition was noted after three days of incubation.

$A_{in}=\pi R^2-\pi r^2$ (mm^2)

A = Area of the inhibition zone
R = Radius of inhibition zone
r = Radius of the disc

Statistical Analysis: The result of anti-diabetic and anti-dandruff activity was tested using Student's t test. The results are expressed as Mean±SD from three independent trials and the P value < 0.05 is significant.

RESULTS

Anti-Diabetic Activity: The anti-diabetic potential of bee venom was tested by comparing the population who were frequently exposed to bee venom (bee keepers) and the population who were not exposed to bee venom (normal population). Blood samples from test population were collected twice i.e. before stinging and after stinging by bees. The levels of blood sugar, triglyceride, cholesterol and total blood count was tested for both the population. The tested parameters were high in the blood samples of control population. The normal range of blood sugar was 70-110 mg/dl. The control population on an average had 151mg/dl which is higher compared to the standard. The blood samples of test population taken before stinging by bees was 104mg/dl on an average of five populations. Similarly, the standard value of triglyceride and cholesterol was 25-160mg/dl and 200mg/dl. The values of control population were 192mg/dl and 237mg/dl which are higher than the values of standard and test population before stinging which ranges around 157mg/dl and 206mg/dl (Figure 1).

On comparing the values of blood sample of test population taken after 30minutes of bee stinging with the values of standard, control and test before stinging, the levels of blood sugar, triglycerides and cholesterol were much reduced. On an average of five populations, the values range around 81mg/dl of blood sugar, 111mg/dl of triglycerides and 189mg/dl of cholesterol (Table1).

Growth Curve of *Malassezia Furfur:* The growth curve studies of *Malassezia furfur* were carried out in SDB. Increase in absorbance was noted with respect to various time intervals at 530nm. The μ_{max} was found to be 0.038hr $^{-1}$ and the doubling time was calculated as 18.23 hrs. The growth curve study was carried out till it attains stationary phase which was attained around 72hrs (Table 2). The exponential phase started after 28hrs of inoculation (Figure 2).

Anti-Dandruff Activity: The anti-dandruff activity of *A. cerana* venom was tested against *Malassezia furfur.* Venom collected by electrical stimulation was dissolved in autoclaved double distilled water and made upto

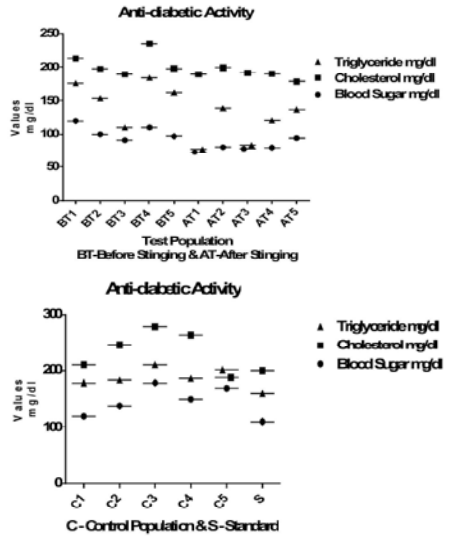

Fig. 1: Graph showing the levels of blood sugar, cholesterol and triglycerides in the populations of Test, Control and Standard

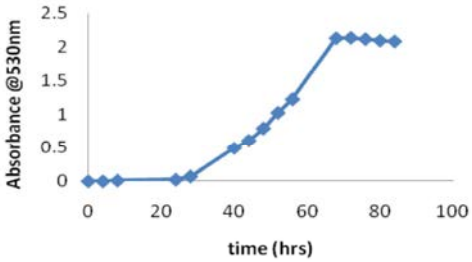

Fig. 2: Graph showing the increase in absorbance of the culture with respect to different time intervals

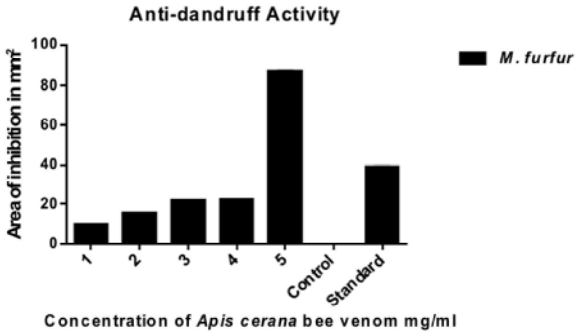

Fig. 3: Graph showing the area of inhibition for different concentrations of Apis cerana bee venom with Control and Standard (Ketoconazole

different concentrations and imbibed in discs of 6mm diameter and placed on plates spread with *Malassezia furfur.* Ketoconazole of 200mg/ml was used as standard

Table 1: Table showing the levels of blood sugar, cholesterol and triglycerides in the populations of Test, Control and Standard

	Test Population (Before Stinging)					Test Population (After Stinging)					Control Population					Standard
Population	BT1	BT2	BT3	BT4	BT5	AT1	AT2	AT3	AT4	AT5	C1	C2	C3	C4	C5	S
Blood Sugar (mg/dl)	120	100	91	110	97	72	81	78	80	94	120	138	178	149	168	110
Cholesterol (mg/dl)	213	197	189	235	198	189	199	191	190	178	210	246	279	264	187	200
Triglycerides (mg/dl)	175	153	110	184	162	77	138	84	121	136	178	183	210	186	201	160

Table 2: Table showing the increase in absorbance of the culture with respect to different time intervals

Time Hrs	0	4	8	24	28	40	44	48	52	56	68	72	76	80	84
Abs at 530nm	0	0	0.01	0.023	0.071	0.495	0.612	0.795	1.025	1.225	2.135	2.141	2.121	2.099	2.087

Table 3: Table showing the area of inhibition for different concentrations of Apis cerana bee venom with Control and Standard (Ketoconazole)

Sl No.	Concentration of Bee venom in mg/ml	Area of inhibition (mm^2) Mean±SD
1	1	10.160±0.0029
2	2	15.433±0.0081
3	3	21.660±0.0057
4	4	21.993±0.0050
5	5	86.900±0.0355
6	Control	0.0
7	Ketoconazole (5mg/ml)	38.833±0.0125

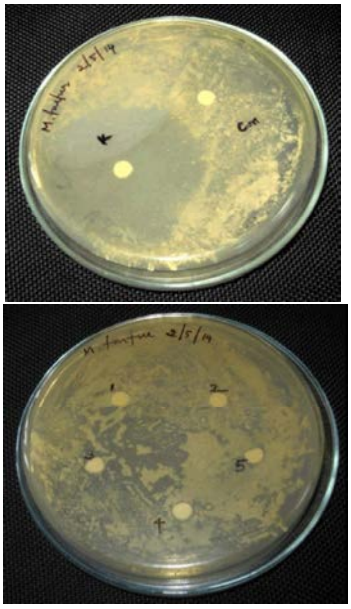

Fig. 4: Plates showing the susceptibility of M. furfur against different concentrations of Apis cerana bee venom with Control and Standard (Ketoconazole)

reference and it showed an area of inhibition of 156.1mm^2. Since venom concentrations were taken from 1-5mg/ml, Ketoconazole was also used at 5mg/ml concentration which showed an inhibition of 38.8mm^2 (Table 3). 5mg/ml venom showed an inhibition of 86.9mm^2 (Figure 3 and 4).

DISCUSSION

Anti-Diabetic Activity: The decrease in sugar level, triglycerides and cholesterol in blood samples of test population after stinging by bees compared to other population and standard may be due to the action of bee venom on insulin level. Melittin, the major constituent of bee venom has an evidence of stimulating the pancreatic β cells to increase insulin secretion thus reducing the blood sugar level [11]. Since the beekeepers are constantly exposed to bee venom by bee stings, the insulin level is kept under control. Also, the action of phospholipase A_2, another major constituent of bee venom is proved to be important for prostaglandin production [12]. Prostaglandins have a strong effect on the maintenance of glucose homeostasis and insulin secretion. Since, phospholipase A_2 is present in bee venom it induces the synthesis of prostaglandin thereby regulating the homeostasis and insulin secretion [13]. Bee venom also increases the glycemic control thereby increasing glucose consumption and reducing lipid breakdown [2]. Cholesterol lowering effect may be due to increased release of cholesterol through liver by the action of bee venom [2].

Growth Curve of *Malassezia Furfur:* Malassezia furfur is a lipophilic yeast and supplementation of lipid substance was found essential for its growth [14]. In the absence of lipid supplementation, no growth was visualized [15]. Corn oil was best suited for its growth. The yeast was cream in colour and oily in nature. There was a long lag phase of 28hours during which it adapts itself for the growth in the media. The exponential phase started around 28hrs and extended till 72 hours after which stationary phase came into effect. The doubling time was calculated as 18 hours which shows that the yeast is slow growing and also the μ_{max} was very less around 0.038 hr^{-1}. Thus, the biomass obtained was also very less.

Anti-Dandruff Activity: Ketoconazole, the standard anti-fungal agent and the chemical used in anti-dandruff shampoos was used as standard reference [16]. Two different concentrations, 200mg/ml and 5mg/ml were tested against *Malassezia furfur*. Of the five different concentration of bee venom, 5mg/ml showed a large area of inhibition of 86.9 mm^2 compared to 5mg/ml of ketoconazole standard which gave only 38.8mm^2. Also, there was a steady increase in the area of inhibition for increasing venom concentration. The inhibition may be due to the action of melittin, a toxic peptide which has been proved to have high anti-microbial property [17]. The mode of action may be based on the lysis of lipid cell wall membrane and thus inhibiting the growth of the lipophilic yeast. This preliminary study has to be extended with animal models to test for any other side-effects.

CONCLUSION

This investigation was done as a preliminary study to check the potential of bee venom against diabetes mellitus and dandruff. Since bee venom reduces the blood sugar and other parameters significantly, a large population will be choosen to check for the detailed action of bee venom on the parameters of the blood samples and also as the bee venom showed good inhibition against dandruff causing organism, animal trials has to be conducted to check its efficacy. Since, most of the shampoos available in commercial market are made of chemicals and have numerous side-effects, bee venom as a natural product when supplemented with natural compounds can be a better cure for dandruff. Thus, value added natural products of bee venom can have a great impact in the near future to control diabetes mellitus, dandruff and various other afflictions.

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