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COLLECTION AND STANDARDIZATION OF HONEY BEE VENOM IN IRAN

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ABSTRACT

Amongst the natural toxins, honey bee venom (HBV) is an ancient remedy for many disorders, particularly arthritis (De Klobusitzky, 1971; Fishkov, 1955; Becker, 1931; Artemov, 1972; Steigerwaldt et al., 1966; Yoshimoto, 1985; Zaitzeff and Poradine, 1973; Beck, 1935). The objective was to evaluate the anti-arthritic effects of HBV, and then to check them with a placebo in a double blind controlled study.

A novel electrical shock device was designed based on a new method of milking consisting of 2-minute intervals between each period of shock. The instrument was patented in Iran under number 24682 on February 4, 1994. A total of 9203 mg of dried HBV has been collected during spring and summer of 1992 and 1993 from 8 hives. The purity percentage of collected HBV was 82.82 ± 8.8 15%.

Based on antimicrobial activity of HBV, the USP disk diffusion microbial assay method (U.S. Pharmacopoeial Convention, 1990) was modified to improve the standardization of the obtained HBV. The standard curve of Sigma grade 1 HBV was linear (r = 0.997294± 0.00246, C.V. % = 3.212± 0.437). The sensitivity of method was 0.15 mg/ ml. Each 1.0576 mg of Obtained HBV was equal to 1.00 mg of the Sigma grade 1 HBV.

Following the collection and standardization of HBV, it will be used for a pharmaceutical preparation to treat some autoimmune diseases. (Key words: honey bee venom arthritis, electrical shock, venom collection, standardization, Bacillus subtilis).

INTRODUCTION

Honey Bee Venom (HBV) has potent anti-inflammatory ingredients like Adolapin, which has anti-inflammatory effects, 70 times more than Indomethacin, and has been known as an antagonist of prostaglandins (Shkenderov and Koburova, 1982). HBV has recently been used for treatment of many diseases, such as Multiple Sclerosis and Osteoarthritis (Scott 1991; Weeks, 1993; Mraz, 1988, 1962; Sommerfield, 1986).

To study the therapeutic effects of HBV it was required to use HBV pharmaceutical preparation. Due to the lack of availability of this preparation in Iran, it was decided to make a pharmaceutical product of HBV.

In order to prepare enough amounts of HBV for producing a suitable pharmaceutical product, we have first planned to make a new electrical shock device for collection of HBV.

Since there was not a suitable published method (presence of many defects in the Cornell method (Benton et al., 1963) and lack of materials with respect to the Mraz method (Mraz, 1962) concerning the standardization of collected HBV, a new method has been developed for this purpose, which is mainly based on anti microbial activity of HBV.

MATERIALS AND METHODS

Eight hives of honey bees (Apis mellifica) with moderate population has been selected for this project. These hives have been fed naturally. During the venom collecting months (June-August) and three months before venom collecting, no anti-mite drugs have been used in them. The hives boxes were standard Langstort ones.

A new electrical shock device has been invented and produced for collection of HBV. Following each alternative shock of 5 minutes, 250 mg dried HBV was collected from each hive. The process of venom collecting has been done once a week during June to August of 1992 and 1993.

Based on anti microbial activity of HBV on Bacillus subtilis (ATCC 6633) (Benton et al., 1963), the USP disk diffusion microbial assay method (U.S. Pharmacopoeial Convention, 1990) was modified to improve the standardization of the obtained HBV. In order to make a suitable microbial suspension for inoculation of Bacillus subtilis, the Simon method (Simon and Vin, 1979) for anti-biotic determination has been applied. The Antibiotic Medium Number 1 from Merck Company has been used as a culture medium for Bacillus subtilis.

Microbial suspension has been estimated by spectrophotometer (Pye- unicam SP6-200). The inhibition zone diameters have been measured by cullis–vernier, 12 to 18 hours after incubation at 36°C.

To sterilize and estimate the purity of collected HBV, Millipore membrane filters (0.2 to 1.2 µ pore size) were applied. An electronic balance (Shimadzu, Libror, precision 0.0001 g) has been used for weighing the filters. The regression analysis method has been applied for statistical calculation of standard curve.

RESULTS AND DISCUSSION

The new electrical shock device for collecting HBV, with the new method of milking has been produced and patented in Iran under number 24862 in February 1994, this Device consists of two electrical and mechanical sections. The electrical section supplies two kinds of electrical current and electrical field. The new method of milking consists of 2-minute intervals between each shock period. Following each alternative shock of 5 minutes, 250 mg dried HBV was collected from each hive. A total of 9203 mg of dried HBV has been collected during Jun- August of 1992 and 1993 (Table 1).

The novel device, which is named bee venom collector, has been registered by the Iranian research organization of science and technology (IROST) as the first one in Iran.

The purity percentage of obtained HBV by this device was 82,72 8.815% (Tables 2 and 3).

Following the making of five standard curves with Sigma grade 1 HBV, the results have been reported in Table 4 and 5 and figure 1.

The results of collected HBV standardization have been reported in Tables 6 and 7.

The result of table 7 show that each 1.00 mg of Sigma grade 1 HBV is equal to 1.0576 mg of obtained HBV.

In fact, the Sigma HBV was lyophilized, but the collected HBV was just air dried. This may explain the mean difference between the two kinds of venoms (0.0576 mg).

In addition, the calculations were based upon weighting the filters, by the electronic balance with precision of 0.1mg. Therefore, the difference (0.0576mg) may also be due to the lack of precision of the balance. It thus may be concluded that there was no significance in the amount of Sigma grade 1 HBV and the obtained HBV.

Also UV spectrophotometric (280 nm) control test has been carried out to establish these results. The spectrophotograms of two kinds of HBV (1mg/ml aqueous solutions) demonstrate that there is no difference between the two kinds of HBV.

Table 1. Amounts of collected Honey Bee Venom (HBV) during spring and summer of 1992 and 1993, from five hives with moderate population.

Milking times	Collected HBV (mg)	
	1992	1993
1th	952	981

2th	983	1285
3th	996	985
4th	955	1096
5th	970	...
Summation of collected HBV during milkings	4856	4347
Mean ± SD	971.2 ± 18.62	1086.75 ± 142.51

CONCLUSION

The collected HBV was comparable with Sigma grade 1 HBV. Thus the collected HBV could be applied for making pharmaceutical products, which is currently under investigation.

Table 2. Amount of collected HBV concentrations used in each test.

No. of Standard curves	F_1 (mg)	F_2 (mg)	A $A=F_1- F_2$ (mg)	B $B =10 – A$ (mg/10 ml)	C $C = B/20$ (mg/ml)
1	3.06	1.06	2.00	8.00	0.400
2	3.18	1.30	1.88	8.12	0.406
3	1.34	1.10	0.24	9.76	0.488
4	2.60	0.68	1.92	8.08	0.404
5	3.70	1.10	2.60	7.40	0.370

F1: Mean Wt. differences of filter membranes before and after filtration of collected HBV.
F2: Mean Wt. differences of filter membranes before and after filtration of sigma standard HBV.
A: Amount of corrected mean wt. differences of the filter membranes.
B: Column B consists of the corrected concentrations of stock aq. Solns. of collected HBV Based upon the milligrams of the passed HBV through the filter membrane in 10 soln.
C: Column c consists of dividing the amounts in column B by 20, to calculate the mg of collected HBV in each ml. of soln.

Table 3. Purity of Collected HBV.

No. of Standard curves	Pure HBV in 10 mg of collected HBV (mg)	Purity percentages of collected HBV %
1	8.00	80.0
2	8.12	81.2
3	9.76	97.6
4	8.08	80.8
5	7.40	74.0

Mean ± SD	8.272 ± 0.881	82.72 ± 8.815

Table 4, Results of evaluation of precision in different days (inter day variations) for standard curves

Corrected Means Values of Inhibitory Zones in Different Experiments (mm)

Concn. (mg/ml)	1	2	3	4	5a	Mean aa	SDaaa	CV%aaaa
0.15	9.25	9.0	8.59	9.50	9.03	9.09	0.33	3.63
0.2	9.68		8.50	9.74	9.51	9.45	0.33	3.53
0.3	10.23		9.43	10.29	10.03	9.98	0.34	3.42
0.4	10.59		9.69	10.66	10.39	10.37	0.28	2.72
0.45	10.70		10.02	10.73	10.52	10.49	0.29	2.76

a number of related standard curves
aa Mean: standard curves character: r = 0.999793, a= 11.53, b =2.97, log C= 0.34 Z.D. – 3.88
aaa Standard Deviation
aaaa Coefficient of variation [C.v.x = (SD/ Mean) . 100]
Mean = 3.212 ± 0.437

Table 5. Result of calculations of logarithmic equations of standard curves based upon the main equation of line.*

No. of standard curves	A	b	Log C= (1/b)Z.D – (a/b)	R
1	11.7946	3.054	Log C= 0.33 Z.D. – 3.86	0.99880
2	11.3872	2.819	Log C= 0.35 Z.D. - 4.04	0.99954
3	10.9249	2.865	Log C= 0.35 Z.D. – 3.81	0.99329
4	11.6983	2.713	Log C= 0.37 Z.D. – 4.31	0.99675
5	11.6189	3.087	Log C= 0.32 Z.D. – 3.76	0.99809

* The main equation of line is Y= a + bx , which is here :

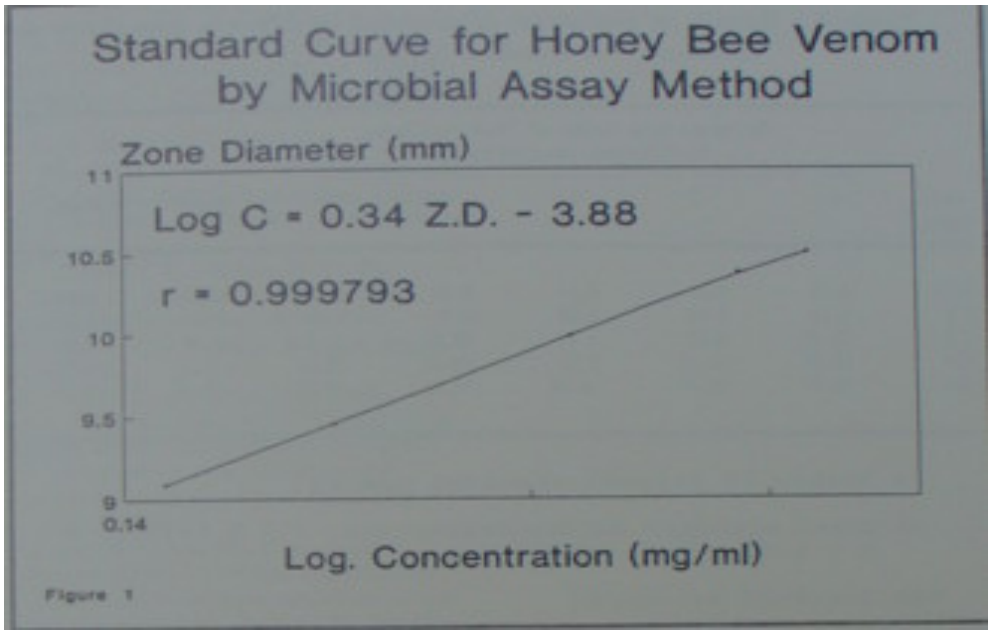

Figure1. Mean of 5 standard curves that have been used for standardization of collected HBV, vs. to Sigma grade 1 HBV.

Table 6. Collected HBV concentrations using standard curves of the related corrected inhibitory zone diameters.

Number Of standard curves	St. C.*Equations And their correlation of coefficients	Corrected Mean value of inhibitory Zone diameters of collected HBV (mm)	Calculated Concn. Of collected HBV based upon St. C.* (mg/ml)
1	Log C = 0.33Z. D. - 3.86 r = 0.99880	10.16	0.438
2	Log C= 0.35Z.D. – 4.04 r = 0.99954	10.62	0.475
3	Log C = 35Z.D. – 3.81 r = 0.99329	10.01	0.494
4	Log C = 0.35Z.D. – 3.81 r = 0.99329	10.68	0.438
5	Log C = 0.32Z.D. – 3.76 r = 0.99809	10.69	0.458

St. c.: standard curve

Table 7. Differences between calculated and corrected concentrations of collected HBV.

No. of standard curves	Calculated concn.of Collected HBV based Upon standard curve (mg/ml)	Corrected Concn. Of collected HBV (0.5 mg/ml)	Differences between Calculated concn. & Corrected concn. Of Collected HBV (mg)
1	0.438	0.400	0.038

2	0.475	0.406	0.069
3	0.494	0.488	0.006
4	0.438	0.404	0.034
5	0.458	0.370	0.088
			Mean = 0.0576*

* The amount 0f 0.0576 mg means that each mg of lyophilized sterilized Sigma standard HBV is equal to 1.0576 mg of collected air dried HBV.

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