Production of Honey Bee Venom and its Anthelminthic Effect

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Abstract

INTRODUCTION

Freshwater fishes upheave maximum production, and household incomes reduce poverty (by fish craft and fishing craft) and enhance nutrition in human welfare more than any fowl or cattle meat product, worldwide. In the current scenario, the biodiversity of freshwater fish is endangered more than any other fauna due to illegal fishing (i.e., using dynamite, electrofishing), refined fishing equipment, overcapacity fish assemblage, defragmentation and degradation of fish habitat, uplifted pollution level, environmental contamination, water abstraction, industrial drainage, new exotic species, changing global climate, etc. Due to one of the above reasons, certain parasites or pathogens habitat in organs of freshwater fishes of Ganga, resulting in elevating mortality rate [1].

The first written records of what are almost certainly parasitic infections come from a period of Egyptian medicine from 3000 to 400 BC, particularly the Ebers papyrus of 1500 BC discovered at Thebes [2].

Parasites are non-independent organisms; either physically or physiologically linked to the host to derive their nutrition: temporarily or entirely for its sustainability, growth, and division might be by exploiting the hosts' existence. This host-parasite relationship is not always species-specific but it is remarkable that the life cycle of parasites can be partial (Intermediate host) or completely (definite host) on Host. They can be ectoparasitic (a parasite on the external surface of the host) or Endo parasitic (a parasite on the Interior of the host). But they invade/infest rapidly. They also have evolutionary adaptations like other organisms. They are omnipresent and independent in any Phylum/ division of plant or animal.

Fish parasitology is one of the popular leading branches in aquatic science which not only involves the study of parasites in fish but helps diagnose diseases in fish, monitoring pollution of water bodies, mortality ratio of fish species census, human pathogens transferred by fish diet [1]. Some of the common fish parasites are – Protozoa, Helminths (i.e., cestode, nematode, trematode) Acanthocephalans, Leech, and Crustacean.

Helminths are of utmost concern, as it is surveyed that between 75,000 and 300,000 helminth species are parasitizing the vertebrates, as a whole. And, almost 20,000 to 30,000 helminth species have ruined the fish industry [3].

As a complementary medicine approach, bee venom has been found to have several biological functions including an antinociceptive effect [4], antibacterial action [5], anti-skin photo-aging [6], and immunity-boosting effect. Several reports have indicated that bee venom administration can induce a significant anti-inflammatory response mediated by the inhibition of inflammation mediators. Bee venom is a rich source of enzymes, peptides, and biogenic amines.

There are at least 18 components in venom that have some pharmaceutical properties, including melittin, apamin, peptide, enzymes (i.e., phospholipase A2), biologically active amines (i.e., histamine adolapin and MCD peptide have anti-inflammatory activities, these substances are present in very small quantities in the whole bee [7,8]. As reports suggested that honey bee venom has numerous pathological effects in the present work we have aimed to study the money bee venom production and its Anthelminthic Effect.

Materials and Methods

Honey bee venom production and collection

Venom was extracted by use of an improved apparatus, containing a finely wired mesh cage surrounding a single transparent glass pane; on which a power supply of 8-10 volts current was provided by Voltameter and the device is kept at an angle of 45°C at the entrance of the artificial bee hives. As a result, bees use to get disturbed and for sell self-defense to sting, (produce venom by venom glands) on glass, which was then collected and stored at 20 ̊C until used. The non-sticky nature of the glathe ss plate allowed the venom to dry naturally so that it could be collected in a pure, powdered form without any contamination. The non-sticky nature of glathe ss plate allowed the venom to dry naturally so that it could be collected in the pure, powdered form without any contamination.

The unit was used twice a day on four selected boxes in rotation, where after 15 minutes boxes were being changed. Once the bees were getting the shock, the power supply was stopped for 10 seconds, to wait for them to recover and sting on the glass plate or the amount of vomit will be more in the collected venom scrape. After that the glass plate was collected and the venom was scraped with the causes of a scraper or blade the scrape is kept inside an Eanpendrof or glass vile and is sealed with the help of tape kept in fresh Ezer, to avoid oxidation.

Samples of honey bee venom were collected from the apiary of the BBAU campus, near the Department of Zoology, where apiculture is going on a small level with around ten boxes, from which four boxes were selected which were having a good population of bees, with eight number of combs in each of them. The variety which is being reared is *Apis mellifera*. Samples were collected by stimulating the bees with electric current pulses, using the 'Honey bee Venom Extractor Unit'.

Parasites collection from Freshwater fishes

Channa punctatus fish species were dissected by bringing them from Specific Sites Gomti U.P. to be brought to the laboratory for examination in the Department of Applied Animal Sciences, BBAU, Lucknow. The standard/body length of fish was measured by using a centimeters scale. The fish was sacrificed by cervical dislocation. After, measuring and weighing the fish were dissected by an incision through the mid-ventral longitudinal line. The stomach, intestine, gall bladder, and liver were examined separately for endoparasites. The stomach and intestine were split open to dislodge any parasites attached to the epithelial lining. Sometimes, the epithelial layers of the stomach and intestine were scraped with a scalpel or brush to remove the parasite anchored. The collected parasite was kept in a Petri plate and after collecting a few numbers of the parasite of

the same species. Just after collection. PBS and HBV (2mg) solution treatment was given to it.

Scanning Electron Microscope-

By this microscope, Ultrastructure was observed in Surface tegumental changes of desired worms by the procedure of Abidi et al. (1998). And it was as follows-

I. **Primary Fixation-** The sample is placed in 2.5% Glutaraldehyde fixative for 2-6 hours at 4˚C.
II. **Washing**- 0.1 % Phosphate buffer is used to wash the sample, for 3 changes each of 15 minutes at 4˚C.
III. **Post Fixation**- 1% Osmium tetroxide is used for 2 hours at 4˚C.
IV. **Washing and Dehydration**- To remove unreactive fixative from the sample again 0.1M phosphate Buffer is used for 3 changes each of 15 minutes at 4˚C as washing.
V. **Drying-** Specimen will be drying by Air drying and Clear Point Drying (Critical Point i.e.,31.5 ˚C at 1100 p.s.i.).
VI. **Specimen Mounting-** Specimen is mounted on to the aluminium stub with carbon tapes.
VII. **Coating-** Sample will coat using Sputter coater to make the sample conductive.

Results and Discussion

Table 1 illustrated the date time and yield of bee venom. **Figure 1** represented the collection of parasites from the intestine of fish and the Cestode of Fish *Channa Punctatus* from the intestine was isolated in **Figure 2** and treated with bee venom. After treating the bee venom. SEM was applied, During SEM, it was observed in **Figure 3** that HBV used, disrupted the parasite tegument. Therefore, this proves one aspect of HBV anthelminthic and antiparasitic efficacy.

Figure 1 Collection of Fish from the Gomti River

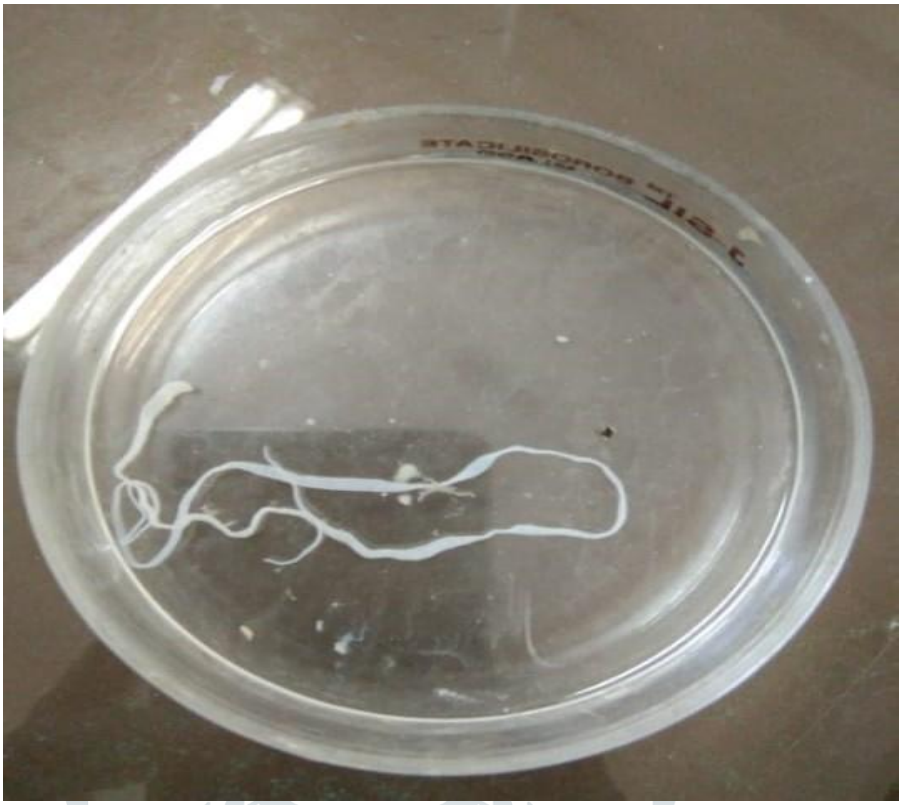

Figure 2 Isolated Cestode of Fish *Channa Punctatus* from intestine Portion.

Disruption of Parasite tegument formed a deep fissure on the integument in some places; on the treatment of HBV to the fluke. This also result in sloughing off and defoliation of integument in some regions and a clear view of the inner membranous layer. It was noted that after HBV treatment fluke remained mortal for 4 minutes on that very day.

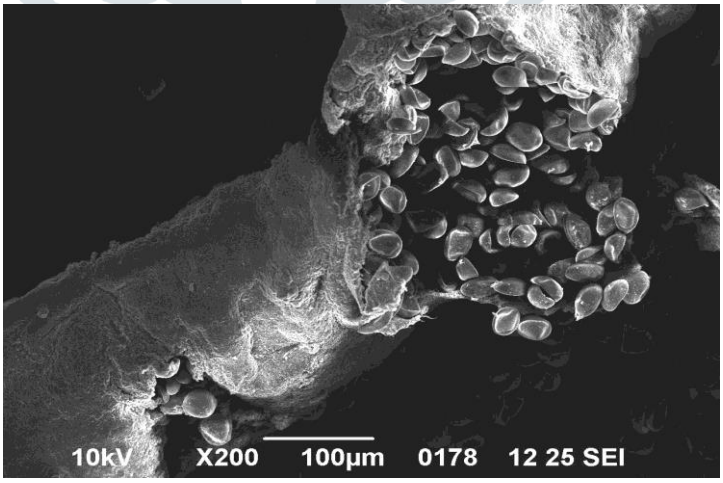

Figure 3 Represents the bursting of Cestode of *Channa Punctatus* fish intestine

Table 1: Represents the collection of honey bee venom.

S.No.	DATE	TIME	YIELD (in grams)
1.	02-02-19	10:30 – 12:00 14:00 – 16:00	0.012
2.	03-02-19	11:00 – 13:00 15:00 – 17:00	0.015
3.	05-02-19	10:00 – 12:00 14:00 – 17:00	0.010
4.	06-02-19	12:00 – 13:00 15:00 – 17:00	0.04
5.	07-02-19	11:00 – 14:00 15:00 – 16:30	0.09
6.	08-02-19	11:30 – 13:30 14:30 – 16:30	0.011

Kim et al. (2014) have studied the antiparasitic effect of bee venom on Trichomonas vaginalis [10]. In this investigation, bee venom effectively has inhibited T. vaginalis growth in a concentration-dependent manner. Adade et al. (2012) have demonstrated that bee venom can affect the growth, viability, and ultrastructure of all *Trypanosoma cruzi* developmental forms, including intracellular amastigotes, at concentrations 15- to 100- fold lower than those required to cause toxic effects in mammalian cells [11]. The ultrastructural changes induced by the venom in the different developmental forms have led authors to hypothesize the occurrence of different programmed cell death pathways. They have established that the main death mechanism in epimastigotes is autophagic cell death, characterized by the presenceautophagosome-likelike organelles and a strong monodansyl cadaverine labeling. In contrast, increased TUNEL staining, abnormal nuclear chromatin condensation, and kDNA disorganization has been observed in venom-treated trypomastigotes, suggesting cell death by an apoptotic mechanism Minieri, L., et al. (1980) [12].

Helminths (parasitic worms) infect more than 3.5 billion people worldwide, causing significant morbidity and economic losses [1,2]. Novel anthelminthic compounds are urgently needed to achieve better control of this important group of parasites given the limited availability of effective vaccines and drugs [3–5].

In a broader way, HBV, an important attribute for the development of a novel anthelmintic, was revealed in the present study with the loss of motility and erosion of the tegumental surface ultrastructures in the HBV-treated worms.

SEM of control worms disclosed intact oral sucker, surface integument, and anas. When HBV treatment was successfully made. Then, it surprisingly results in the erosion of the integument more over the regions of the oral sucker with a deep fissure on it and slogging off and defoliation of the integument surface. It has been reported that HBV disrupts membrane integrity by altering membrane potential and permeability, causing blebbing of the plasma membrane ultimately leading to apoptotic events [13].

Conclusion

Honey bee venom (HBV) has been used for the treatment of various ailments but limited information is available on its anthelmintic properties. To investigate the anthelmintic properties of HBV, Nematode from *Channa panctatus* was drawn out from carnivorous fishes of River Ganga and examined In vitro for histo morphological changes to be evident as proof of this experiment.

Here, the control worm (no treatment with HBV) was considered for comparison with the worm treated with HBV separately. With the aid of SEM ultrastructure of the worm revealed deep fissures in many regions of the integument along with slogging off and defoliation of the same with complete damage to the worm integument. Assuring, with anthelminthic properties at the end.

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