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Anthelmintic and Food Supplementary Conscientiousness of Apitoxin in Poultry Model

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Abstract

The current investigation was conducted to analyze the anthelmintic and food supplementary activity of bee venom in poultry model. The crude apitoxin was collected by venom extractor (invented and developed by the scientists at University of Allahabad, India) from Indian honey bee, Apis indica, reared at work station by the application of mild electric shock. It is a shiny liquid dries up quickly when come in contact of air and hydrophilic in nature. Chemical constituent of apitoxin includes peptides as melittin, apamin, phospholipase-A2, hyaluronidase, histamine, heptapeptides, adolopin and apamine were separated and characterized by High Performance Liquid Chromatography (HPLC) and Mass Spectroscopy (MS). The anthelmintic and food supplement activity of different fractional doses of toxin was analyzed in vitro and applied in vivo in well designed control and experimental poultry bird models. The control group was kept on plain poultry feed, whereas the other four groups were supplied with various fractions of apitoxin to poultry feed. The average daily body weight was found to be increased for model supplemented with honey bee venom in comparison to control poultry model. The findings were substantiated and level of significance was calculated by biostastistical applications. Simultaneously on the other hand in vitro bio assay of bee venom against heterakid roundworms, recovered from same model were tested and applied in vivo in experimental poultry models. The constituents of bee venom (heptapeptides, 7Kda) play a significant key role in anthelmintic activity against heterakid nemic fauna of avian vertebrates. These finding gave an idea about better and managed poultry through supply of apitoxin in their daily diet that acts as food supplement as well as anthelmintic factor for newly born chicks and adults.

Keywords: Apitoxin, *Apis indica,* Food supplement, Anthelmintic, Poultry, HPLC.

Introduction

Honeybees are the earliest known social insects to man. They have survived alongside their ever-changing environment for 120 million years. They are recognized and appreciated as the single most important insect pollinators and thus, increase the productivity of food. Bee venom is an intricate combination of proteins, peptides and low molecular components. Already in the early ancient civilizations know about the healing found virtues in the painful bee stings. Bee stings are probably one of the first natural cures for arthritis. In the ancient civilization of China, India, Egypt, Babylon and Greece bee venom was used for apitherapy[1]. The modern uses of bee venom in apitherapy were initiated through the efforts of Austrian physician Philip and describe the peculiar connection between the bee stings and Rheumatism[2]. The venom gland of honey bee comprises two glands are located in posterior portion of the abdomen and sting apparatus is a modified form of ovipositor. It consists of a secretary filamentous region, connected to a reservoir at its proximal portion, in which the venom is stored[3,4]. The workers sting only once, which leads to their death. Venom contains 88% water. At least 18 pharmacologically active components have been described so far; including various enzymes, peptides and amines[5]. Bee venom therapy which utilizes the application of bee venom to treat various diseases has been used since ancient times in traditional medicine[6-8]. A mature defender or forager contains about 100-150 µg of venom, and it inject 0.15 – 0.30 mg of venom via its stinger, a honeybee can inject 0.1 mg of venom via its stinger[9]. A large number of studies have been carried out on the composition of honeybee venom. Much of the basic identification of compounds, their isolation and the study of their pharmacological effects of bee venom was done in the 1950's and 1960's. There are some comprehensive summaries in about the pharmacological effects of bee venom and allergies to the Hymenoptera venom of bees, wasps and ants were worked out by the earlier scholars and give a very good overview of its composition, patterns of harvesting, effects and activities[10-12]. There are several earlier literatures are available about anti-inflammatory, anti-rheumatic, anti-hypertension and anti-bacterial effect of *Apis indica* venom but the anthelmintic and food supplementary conscientiousness of apitoxin is scarce[13-16]. For this reason in the present investigation author try to explore the significance of Indian bee venom as natural bio-product to cure the helminthes infection and food complementary activities in poultry model. Poultry is the basic source of healthy meal and also play major role in the economy of country. But it is suffering now a day and poultry formers loss their economy due to helminthes infection and unfair growth of broilers.

It was supposed that helminthes infection reduced the egg laying capacity in layers and growth of broilers. Not only due to these activities but also several variety of helminthes infection have zoonotic potential and their transmission may be up to highest trophic level of the food chain means human being through the edible matters, like poultry flesh. Therefore, author tries to resolve these issues by the application of apitoxin, a natural bio-product of Indian honey bee, *A. indica*.

Materials and Methods

The poultry were reared in the village Satnaraini, Khaga, district Fatehpur, Uttar Pradesh, India during 2012 to 2014 under the supervision of author and poultry experts of University of Allahabad (A Central University), Allahabad, Uttar Pradesh, India. However, rearing or culture of *A. indica* (Apiculture) was performed in Collectorganj, District Fatehpur. The crude bee venom was collected from Indian honey bee on site with the help of bee venom extractor (Figure-1A). An electric shock of weak strength was applied in venom extractor. Resultantly honey bee irritated and left a drop of bee toxin on glass plate attached to venom extractor. Dropped venom scrapped out and stored at -18ºC. The collected bee venom was lyophilized that gave rise powdery form of it, known as crude bee venom (Figures-1B, C). Simultaneously the stool of poultry was critically observed under stereomicroscope to diagnose the helminthes infection[17,18].

The different concentration of crude bee venom was tested against parasitic helminthes of model organism *in vitro* and *in vivo* with experimental poultry models. In the mean while, food supplementary activity of bee venom worked out in same poultry model by standardized experimental designing. There was a five set each with five models in which four were treated as experimental and one as control set. The qualitative estimation of honey bee venom (Apitoxin) was done by the Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) and pharmacologically active compounds in bee venom was detected and characterized by the application of HPLC and mass spectroscopy[19].

Results and Discussion

The honey bee venom collected from the Indian honey bee *A. indica* was processed and components in crude extract identified by the SDS-PAGE (12%). It was found that bee venom contains variable number of high molecular weight compounds (Figure-2). The individual constituents of crude bee venom were characterized by the application of High Performance Liquid Chromatography (Figure-3). It was noticed that the crude bee toxin contains several proteins, peptides, biogenic amines, amino acids, sugars, volatiles and minerals. The details of constituents detected during investigation with their respective dry weight percentage were summarized in Table-1. The respective accomplishment of crude bee venom fractions and their relative effects were noticed and hypothesized by the author in the present investigation after critical analysis and it was found to be significant (Table-2).

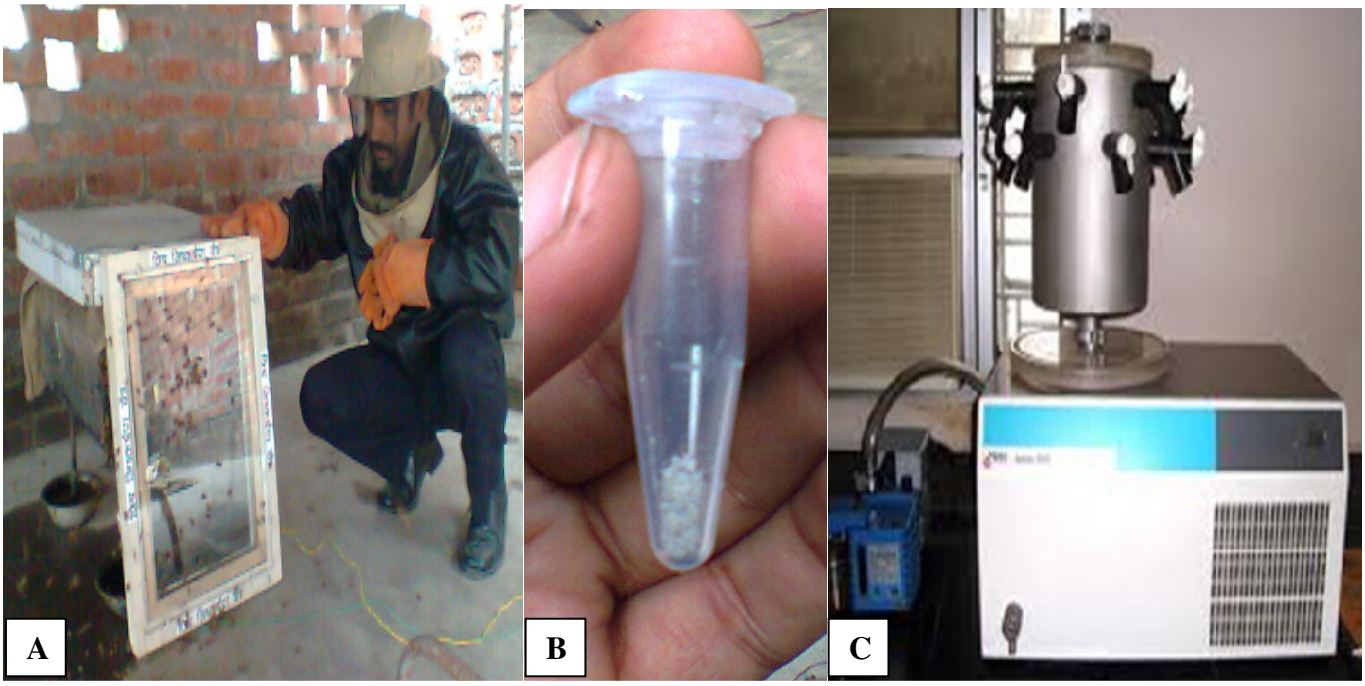

Figure-1
On site bee venom extraction with the help of venom extractor (A); collected crude honey bee venom in eppendorf after lyophilization (B); lyophilization or freeze drying machine, the Lyophilizer (C)

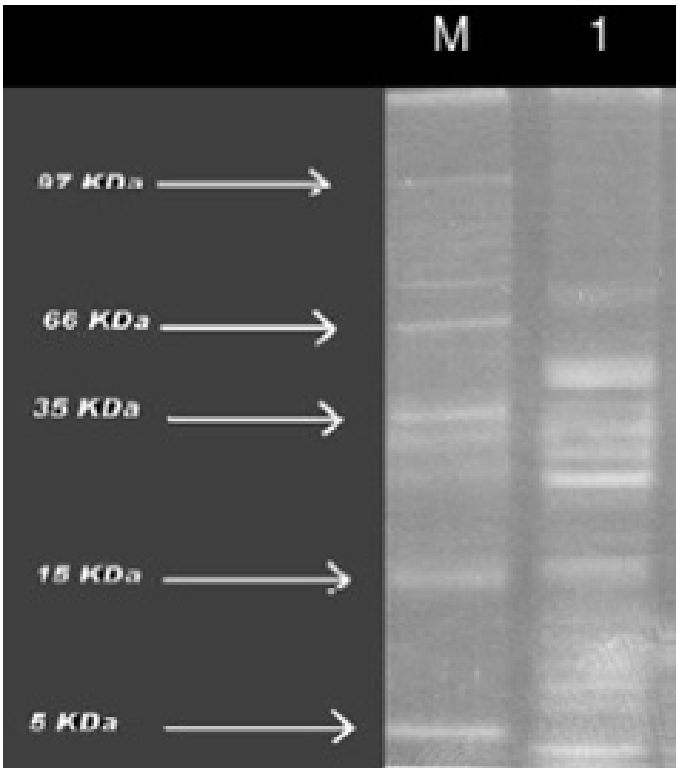

Figure-2

Gel photograph of SDS-PAGE (12%) with standard molecular marker, protein ladder1KDa (lane M) to know the presence of different proteins in apitoxin of *A. indica* (lane 1)

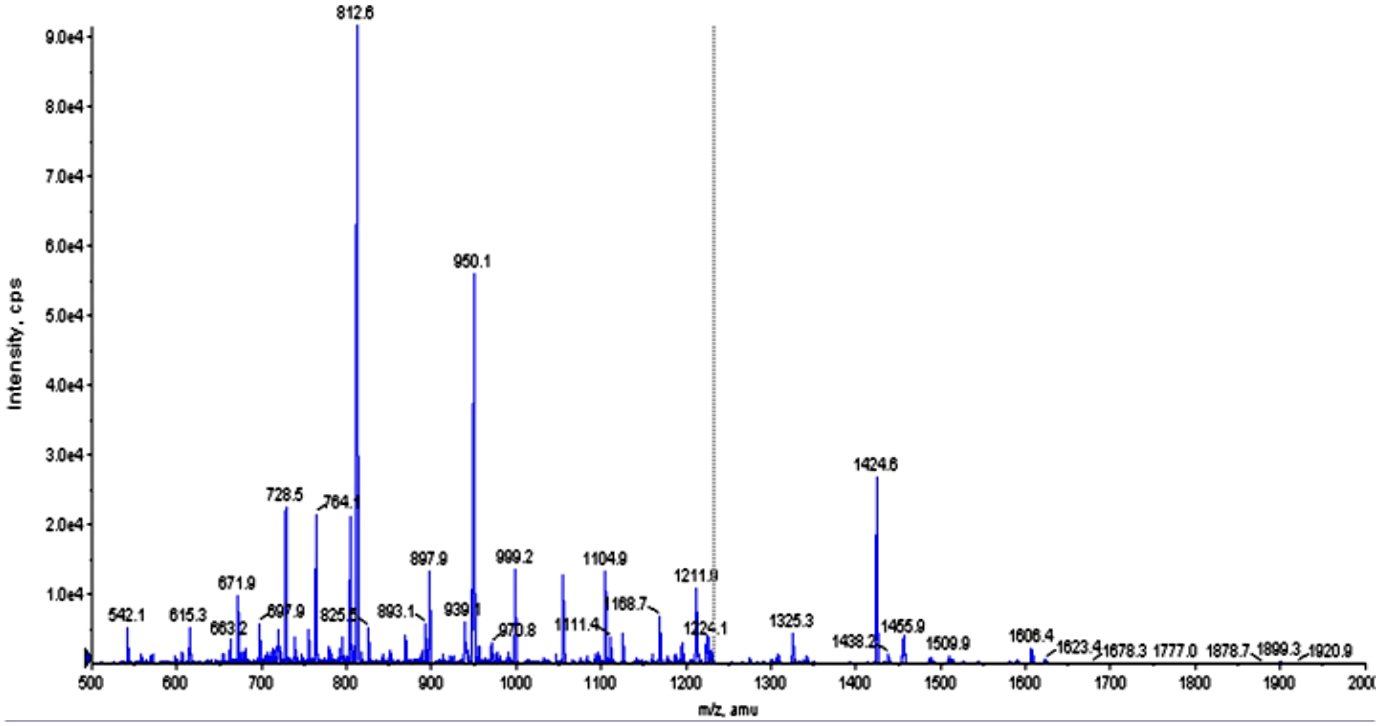

Figure-3

Graphic representation of *A. indica* apitoxin components and their respective intensity after HPLC (High Performance Liquid Chromatography)

Table-1
Composition of crude honey bee venom extracted from *A. indica*.

Substance Group	Component	% of dry weight
Proteins (Enzymes)	Phospholipase A2	10.3-11.9
	Hyaluronidase	1.5-2.5
	Phosphatase	1.0-1.3
	Glucosidase	0.5-1.0
Peptides	Melittin	38.5-49.5
	Apamine	2.0-3.3
	Secapine	0.5-2.0
	Adolapine	0.5-1.2
	Procamine	1.0-2.0
	Protease inhibitor	0.1-0.8
	Tertiapine, Cardiopep	1.0-2.0
Biogenic amines	Histamine	0.5-2.2
	Dopamine	0.3-1.0
	Noradrenalin	0.1-0.5
Amino acids	Aminobutyric acid, α-amino acids	1.5-2.0
Sugars	Glucose, Fructose	2.0-4.5
Volatiles (pheromones)	Complex ethers	3.5-8.0
Minerals	P, Ca, Mg	3.5-4.5

Table-2
Components of crude honey bee venom extracted from *A. indica* their accomplishment and respective special effects

S. No.	Fraction	Accomplishment	Special effects
1	Mellitin (about 50 % of dry bee venom)	Anti-bacterial and anti-inflammatory and growth promoter properties. Stimulates ACTH secretion in pituitary gland.	High anti-inflammatory effects; long-term, quick- acting histaminic effects: edema, thermal augmentation, itching, ache; anti-oxidant action.
2	Apamin (small basic peptide)	Improve central and peripheral nervous system effects, secretion of serotonin and dopamine.	Increases the threshold of central and peripheral pain hence decreases pain, augmented sense of well-being.
3	Adolapin	Inhibits enzymes in metabolic cascades of arachidonic acid.	Anti-inflammatory and analgesic (pain alleviation) action.
4	Hyluronidase, Secapin, Procamine	Inhibition of pain substance.	Anti-inflammatory, pain reducing.
5	Phospholipase A2, Heptapetidase	Growth promoter and anthelmintic.	Stimulate the growth and body mass; reduced the helminthes infection by the inhibition of parasitic metabolism.

The assessment of food supplementary and anthelmintic potential of crude apitoxin was worked out by the three months continuous investigation in well standardized and hygienic environment. There were five sets of day old poultry chicks, each set carry five individual taken in consideration. Every individual of each set was treated with different doses of crude bee venom (lyophilized) except one set, the control group. Everything (dietary materials and local environment) provided to model organism either control group (CG) or experimental groups (EGA, EGB, EGC, EGD) in same quality and quantity. The doses of crude bee venom during experiment were given in µg/100g of feed/kg of body weight unless otherwise stated. The individual of controlled group was orally fed by 0.0µg/100g/kg while EGA, EGB, EGC and EGD by 250, 500, 750 and 1000µg/100g of feed/kg body weight. The fortnightly augmentation in growth of poultry as increase in body mass was recorded continuously for three months. The average growth rate was recorded as 46 (37-59) g/kg in EGA; 73 (65-83) g/kg in EGB; 111 (89-122) g/kg in EGC and 82 (72-93) g/kg in EGD in every fortnight. The optimum and peak growth (Figure-4) was notice in experimental group C (EGC).

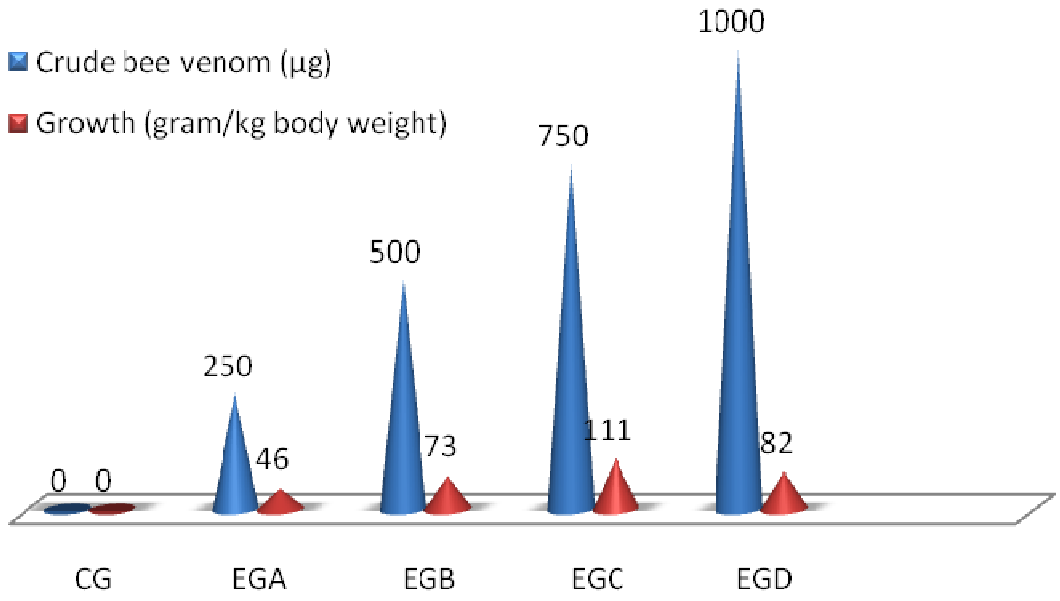

Figure-4
Correlation of increase in body weight vs crude bee venom doses in poultry model

For the assessment of anthelmintic potential of honey bee venom (BV) five set of experimental design were established. All the twenty five poultry birds taken in account were dehelminthised by the oral anthelmintics and confirmed by the stereomicroscopic stool analysis. After that all the birds were orally fed by the equal dose of heterakid eggs (about 100 eggs/ml of water). The establishment of nemic infection was analyzed weekly and confirmation of infestation announced after stool analysis that carry eggs or larvae and anal itching behavior. Latter on each group EGA, EGB, EGC and EGD was treated with 250µg/kg, 500µg/kg, 750 µg/kg and 1000µg/kg oral dose of lyophilized crude bee venom respectively except control group (CG). The level of infection was assessed periodically at the interval of seven days for three months. During the period of investigation in was observed that the crude bee venom have negative impact on the establishment and pattern of infection in each group treated with bee venom. It was numerically found to be significant in experimental group D (EGD) because of negligible heterakid nemic infection after completion of experiment.

The composition of fresh and dried BV differs mainly in regards to the volatile components but the overall biological activity is similar. Since the ancient civilization it was noticed that the painful bee stings have a healing impact to wounds. The bee venom extracted was a transparent liquid dries up easily even at room temperature, odorless, water soluble, ornamental pungent smell, a bitter taste, hydrolytic blend of proteins with basic pH that is used by bees for defense and similar to the finding of earlier investigations[20,21]. When it comes in contact with air it forms grayish-white crystals. The main components of toxicological significance are melittin (50%) and phospholipase A2 (10%) of which melittin is the dominant toxic component[22]. Usually melitttin, apamine and phosphorylase A_2 have been the known chemical constituents of bee venom on which most of the studies have focused in recent years. The present investigation yielded fractions of 7KDa (heaviest molecular weight, hitherto unknown) showing potential anthelmintic characteristics are peculiars. The findings of the present investigation was supported the literature in which it was noticed that the polymeric nanoparticles of arthropods bio products acts as a potential anthelmintics in common domestic goat, *Capra aegagrushircus* against strongylid parasitic roundworms[23]. It was also observed that the finding was corroborated with[24] and they have described the impact of different concentration of freshly collected bee venom against parasitic helminthes of model organism *in vitro* and *in vivo*. By the experiment it was revealed that the fraction of honey bee venom has a potential food supplement, anthelmintic and anti-bacterial activity[25].

Conclusion

The anthelmintic and food supplementary conscientiousness of several fractional doses of apitoxin were analyzed *in vitro* and applied *in vivo* in well designed control and experimental poultry bird models and found to be significant numerically. The present investigation yielded fractions of 7KDa (heaviest molecular weight, hitherto unknown) showing potential anthelmintic characteristics are peculiars so far. Therefore, the food supplementary and anthelmintic potential of crude honey bee toxin for health improvement of poultry birds could results from conservation of this natural product and supposed to be a source of new drugs development for the treatment of neglected helminthes diseases and redundant growth of economically important animals such as poultry birds.

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