

Morphological, Histological Changes and Acetyl Cholinesterase Activity in Chicken Embryos After Exposure to Abamectin Insecticide

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Abstract

Abamectin is a bio-insecticide, derived from the soil bacteria *Streptomyces avermitilis*. This insecticide is used in public health and agriculture to protect crops. Major adverse impacts of Abamectin are neurological symptoms acting on the peripheral nervous system. The aim of the current study is to reveal the toxic effects of Abamectin on chick embryo Ross 308 including morphological and histological changes and acetyl cholinesterase activity. 120 fresh fertilized eggs were divided into 6 groups; two of them were used as control. After 2 days of incubation, the eggs were injected with 100 µL of Abamectin solution (diluted at concentrations 360, 540, 900 and 1800 ppm) into the yolk sac. The Results showed that the mortality increased significantly in chicks treated with Abamectin, but had a lower weight in comparison to the control groups. Treated chicks started hatching at day 22 but were physically weak with drooping limbs, paralysis and then died after 24 hours of hatching. Some chicks did not normally hatch and needed assistance. They characterized by limb defects, failure retraction of yolk sac with bleeding. Histological examination of the liver showed hepatic cell degeneration, congestion in the central vein, infiltration of inflammatory cells and hepatocytes necrosis. Furthermore, the Acetyl cholinesterase enzyme analysis showed a significant decrease in the enzyme activity which leads to inhibition the activity of the body systems. It is concluded that low and high concentration of Abamectin has adverse impacts on chick embryo by changing some of morphological, histological characteristics and acetyl cholinesterase activity.

Keywords: Abamectin, Acetyl Cholinesterase, Morphological, Chick Embryo Ross308

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I. INTRODUCTION

Abamectin (AM) is a bio insecticide, used in public health and agriculture to protect citrus, pears, apples, fruits, potatoes, tree nuts and different of other vegetables (Akashe *et al.*, 2018). AM derives of avermectin from the soil bacteria *Streptomyces avermitilis*. It belongs to a general class of closely related macrocyclic lactones and consists of two components including major avermectin B1a (CAS No. 65195-55-3) and avermectin B1b (CAS No. 65195-56-4) which in turn consists of about 80% and 20% respectively. Both B1a and B1b possess a structure of 16-membered ring (figure 1) (Takahashi *et al.*, 2002; Jayakumar, 2009).

The toxicity effect of Abamectin occurs as a result of the interaction between these of two active components (Yoon *et al.*, 2004; Campbell, 2012). Major adverse impacts of Abamectin are neurological symptoms acting on neuron receptor gamma amino butyric acid (GABA) in the peripheral nervous system (Raftery and Volz, 2015; Novelli, 2016). These effects are mydriasis tremor/convulsion through the

interaction between GABA-ergic and hyperpolarization of muscle cells/nerve.

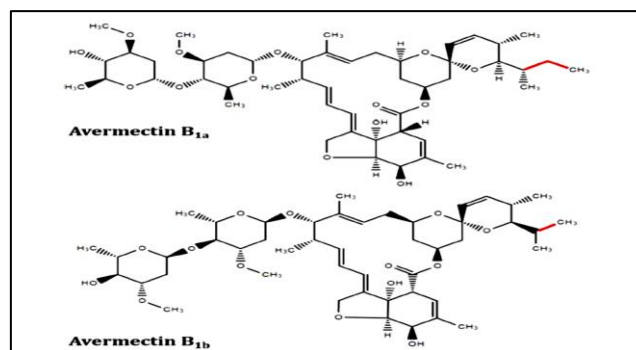


Figure1: showing chemical structure of Abamectin: Abamectin as structure consists of natural avermectins B1a and B1b. B1a compounds of ethyl group while B1b has a methyl group attached in the ring (Terali *et al.* 2018).

Neither genotoxicity nor developmental neurotoxicity, reproductive toxicity and carcinogenicity were observed. Pesticide affects neuron transmitter Acetylcholine by

inhibition cholinesterase enzyme activity and then leading to accumulation on neuromuscular synaptic and occurrence neurotoxicity (Wilson *et al.*, 2005; Bjorling- Poulsen *et al.*, 2008). Acetyl cholinesterase inhibition is a bio indicator for exposure toxic pesticides (Abass, 2014; Fossati *et al.*, 2015), which leads to physiological weakness (Sardar *et al.*, 2020) neuronal degeneration (Kennedy *et al.*, 2014; Thiripurasundari *et al.*, 2014) and changing behaviour in *Oreochromis mossambicus* (Kushwaha *et al.*, 2020). The exposure to insecticides is associated with inhibition of acetyl cholinesterase in the central nerve system especially in the brain (Wilson *et al.*, 2005) due to the lipophilic of Abamectin properties (Khanna *et al.*, 2002; Novelli *et al.*, 2012). Only one study refers to the decrease Acetyl cholinesterase (also known as Ach E) activity in tissue brain to 48 – 96 % in 26 species wild birds, among them *Gallus gallus domesticus* (Bang *et al.*, 2019). The chicken meat is a source of protein for human-being in many different countries. Poultry feed may contain residues of pesticides, which accumulated in food chain down to terminal consumer (Koc and Karakus, 2011; Salar-Amoli and Ali-Esfahani, 2015). The chick embryo is an excellent model for toxicological research (Kotwani, 1998). Therefore, the current study aimed to reveal morphological, Histological changes and Ach E activity after exposure to Abamectin insecticide in chick embryo.

II. MATERIALS AND METHODS

The current study used the commercial biocide Abamectin 1.8 % EC (Emulsifiable Concentrate), product from King Quesson Industry Co., Ltd in China. The stock solution of insecticides was diluted to prepare four concentrations of the insecticide 360, 540, 900, and 1800 ppm (Part per million).

A. Experimental Design

120 Fresh fertilized eggs of chick broiler Ross 308 were purchased from Rison Company in Erbil city. Eggs were weighted, 65-68 gram and then cleaned by cotton and Ethanol 70 % to remove any contamination on the egg shell. The eggs were then divided into six groups, 20 eggs for each including the control groups negative (normal) and positive were injected with distilled water. After that, the eggs were incubated at temperature $37 \pm 0.5^\circ\text{C}$ and relative humidity 70-75 %. After two days of incubation, the eggs were injected with single dose $100 \mu\text{L}$ according to (Chaudhary, *et al.*, 2017) for each concentration 360,540, 900, and 1800 ppm, into the yolk sac by using 0.3 mL syringes with fine needle. Injected eggs were then closed by adhesive tape to avoid the contamination.

B. Histological Study of the Liver

The liver of chicks was quickly removed and fixed in formaldehyde solution 10%, and then washed in different concentrations of the ethanol. Tissues were dehydrated and then embedded in paraffin wax and later sectioning using the rotary microtome Leitz 1512 MH340/German. Sections were cut at $6 \mu\text{m}$ in thickness then staining with Haematoxylin and

Eosin (H&E). Tissues of the liver were photographed by microscope digital camera SCMOS- 05000KPA.

C. Preparation of Samples and Enzyme Analysis

After 21 embryonic days of incubation at about a stage of 45 according to (Hamburger and Hamilton, 1951) brains of the treated chick embryo were removed by a scalpel and then stored at -20°C . Brains of chick embryo were homogenized by crushing mortar and mixed with 3ml distilled water and 3ml buffer solution. According to (Alias and Mohammad, 2005; Mohammad, 2007), buffer solution was prepared on pH 8.1 and consisted of 0.19g Sodium Chloride, 4.38g Sodium Barbital and 0.02g potassium di-hydrogen phosphate from Sigma Aldrich were dissolved in 125 ml distill water. By using pH meter (PHS-3BW) was measured changing pH 1 of the homogenate brain solution (HBS), then add 0.2 ml acetylcholine iodide 7.1% (Substrate) to the HBS for measured the pH 2. While, the pH of blank solution was measured by adding 3ml distill water, 3ml buffer solution with 0.2 ml substrate 7.1%. The changing of pH (ΔpH) was calculated as follows: $\Delta \text{pH} = (\text{pH}_1 - \text{pH}_2) - \text{pH blank}$.

D. Statistical Analysis of Data

The data of weights of chick embryos and Acetylcholinesterase analysis was calculated by Statistical Analysis System (SAS) Completely Randomized Design. One way ANOVA, Duncan Multiple Range. The values showed significant decrease $p < 0.01$ and $p \leq 0.05$.

III. RESULTS

The current study focused on chick age 21 day of incubation to investigate the morphological and physiological effects of Abamectin insecticide. As mentioned above, four concentrations of this insecticide (360, 540, 900 and 1800 ppm) were used by injection the yolk sac with $100 \mu\text{l}$ of the insecticide. On day 21 of incubation, chicks of control groups (negative and positive) were normally hatched and physically active (figure 2A). On the other hand, chicks of treatment groups at concentrations 360, 540 ppm/egg were delayed in hatching to day 22^{ed} of incubation. They were physically weak, panting with droopy wings, weakness of hind-limbs and cannot normally walk. Chicks also showed complete paralysis, coma and they died after 24 hours of hatching (figure 2B and C). Eggs treated with 900 and 1800 ppm/egg did not normally hatch and they needed assistance. Treatment with concentrations 900 ppm/egg led to failure to retract the yolk sac, adjoining legs and arched toes (figure 2D). Similarly, Abamectin at concentrations 1800 ppm caused splayed legs with curved toes, delayed yolk sac retraction with hemorrhage (figure 2E).

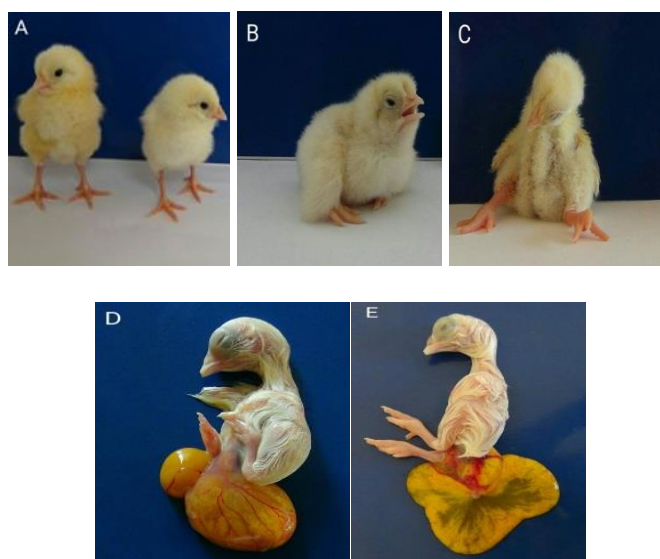


Figure 2: (A) represents control group. B, C, D and E chick treated with 360, 450, 900 and 1800 ppm of Abamectin respectively.

As it is shown in table 1, the current study also showed a significant increase in the mortality in chicks treated with Abamectin in comparison to the control groups. The mortality was 65% in embryos treated with 360 ppm, while the higher percentage was 90% in embryos treated with 1800 ppm compared to negative (5%) and positive (10%) control groups. Moreover, the control average weight was (48g and 46g) for negative and positive control groups respectively, while injected the chick embryos with Abamectin had a lower weight compared to the controls (figure 3).

Table 1: Showing mortality percentage and weights of embryo Ross 308 in experimental groups.

Experiment group	Doses/egg	No. of egg treatment	No. of dead embryo	Mortality %	Weight of embryo (g) Mean ± SE ^(a)
Group A	0	20	1	5	48.0±1.20**
Group B	100	20	2	10	46.0 ±1.45*
Group C	360	20	13	65	38.0 ±1.15*
Group D	540	20	15	75	34.6 ±1.76*
Group E	900	20	17	85	32.0 ±2.64*
Group F	1800	20	18	90	29.3 ±3.17*

(a) The Values weight of embryo are expressed as Mean ± SE (standard Error) were showed significant decrease on **p<0.01 , *p ≤ 0.05

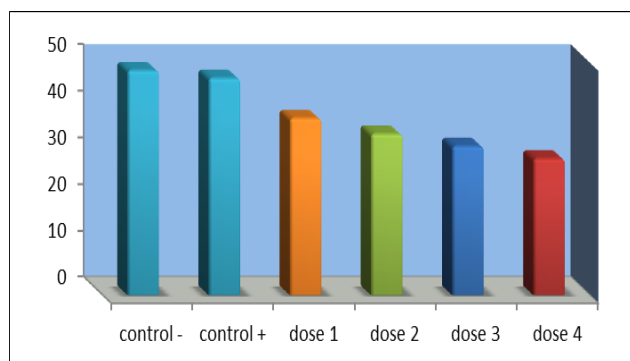


Figure 3: Demonstrates a significant decrease in the weights of treated chick embryo Ross 308 compared to the control groups.

Microscopically, sections in the liver of control chick ED 21 showed normal architecture in the liver cells, normal sinusoids and normal central vein (figure 4 A and B). Briefly, histological sections in the liver of chick embryos treated with 900 and 1800 ppm (figure 4C,Cc and D,Dd) of Abamectin on ED 21 observe dilatation and congestion in the central vein and sinusoidal space necrotic cells. In addition, there was hepatic cells degeneration as well as infiltration of inflammatory cells.

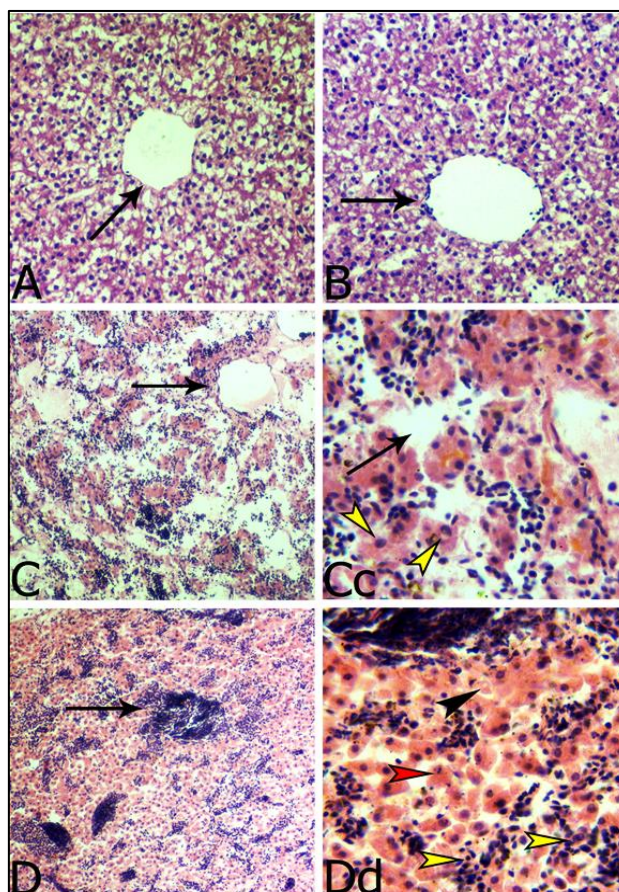


Figure 4: showing histological sections in the liver of chick embryos on 21-22 day of incubation. (A and B) sections of control groups B, injected with distilled water. The control groups show normal structure of the hepatocytes around the central vein (black arrow). (C and Cc) sections magnification

(10X and 40X respectively) in the liver treated with 900 ppm of Abamectin on ED 21 show dilatation in sinusoids with bleeding (Black arrow) and vacuolization (yellow head arrow). (D and Dd) sections magnification (10X and 40X respectively) in the liver treated with 1800 ppm of Abamectin on ED 21 observe congestion of central vein with infiltration of inflammatory cells (black arrow). It can be also seen dilatation in blood sinusoid with necrosis (red head arrow) vacuolization (black head arrow) and infiltration of inflammatory cells (yellow head arrow). (Section stained by Haematoxylin and Eosin).

The current study also revealed the activity of Acetyl cholinesterase (Ach E) in chick embryo after treatment with Abamectin insecticide. As shown in table 2, the Ach E analysis decreased significantly in enzyme activity at percentage 73%, 87%, 90, 97% respectively due to 360, 540, 900 and 1800 ppm of insecticide respectively.

Table 2: showing changing of values pH (Δ pH) and inhibition percentage of Ach E activity in chick broiler Ross 308.

Experimental groups	Doses/ppm	Δ pH Mean \pm SE	Inhibition% ^(a)
Croup A	_____	0.86 \pm 0.01	0
Croup B	100	0.86 \pm 0.01	0
Croup C	360	0.23 \pm 0.21	73
Croup D	540	0.11 \pm 0.90	87
Croup E	900	0.085 \pm 0.55	90
Croup F	1800	0.025 \pm 0.15	97

^(a) Inhibition % = Δ pH control – Δ pH treated/ Δ pH control * 100

The values of Δ pH expressed significantly decreased $p \leq 0.05$.

IV. DISCUSSION

Pesticides are chemical or biological compounds used in agriculture to protect crops from the pest and, in veterinary, to prevent the spread of diseases carrying parasites insect (Gul *et al.*, 2017; Akashe *et al.*, 2018) Abamectin 1.8 % EC is a bio insecticide available in commercial markets with different names. Therefore, the current work was carried out to investigate the effect of this insecticide on chicks at 21st day of incubation. Chick embryos are a good model for studying the toxicity of chemical, because they are easy to manipulate and their development is short and may homolog genomic structure with higher vertebrates. These reasons made the chick as a perfect model for studying changes morphogenetic (Stern, 2018) and neurobehavioral (Abdul-Gani *et al.*, 2012).

The liver is an effective organ in removing the toxic effect of many substances and particles that enter the body, as well as removing vital detoxification and xenobiotic of organs (Hernandez *et al.*, 2013). Exposure to insecticides affects the liver function and activities enzyme Alanine aminotransferase (ALT), Aspartate amino -transferase (AST) which leads to liver tissue damage (McGill, 2016). A high level of ALT and AST enzyme in serum or plasma causes hepatotoxicity and leakage of lysosome enzyme (Chaudhary *et al.*, 2003). The elevation in the level of ALT, AST is associated with histopathological lesions like degeneration of

hepatic cell, enlargement dilation of sinusoids and vacuolar degeneration (Ksheerasagar *et al.*, 2011). Many of studies revealed a significant increase in the ALT and AST enzymes after exposure to insecticides (Farag *et al.*, 2016; Kushwaha *et al.*, 2020). Moreover, exposure to insecticides induces oxidative stress and production of reactive oxygen species (ROS) which in turn increases the free radical and occurrence damage of macromolecules such as proteins, lipids and DNA damage (Agrawal and Sharma, 2010; Sharma and Sangha, 2014). The exposure to insecticides is associated with histopathological alteration in liver tissues such as necrotic changes and damaged of hepatocytes (Ahmed *et al.*, 2020; Basal *et al.*, 2020).

Abamectin plays on Ca-Mg ATPase enzyme and decreases of intracellular Calcium level which in turn leads to inhibition of Ach E activity and accumulation Acetylcholine (Bradberry *et al.*, 2005; Lotti, 2010). Furthermore, Abamectin affects GABA receptor by activation of gated chloride channels, which allows chloride ions flows to intracellular then caused hypopolarization of muscles (Xu *et al.*, 2017; Srivastava *et al.*, 2020). Basically, the inactivation of Ach E leads to twitching of muscles and causes tetanus and paralysis of muscles (Singh *et al.*, 2004). The current study revealed that treatment of chick embryos with Abamectin at does 360 and/or 540 ppm causes droopy wings as well as splayed legs, which led to paralysis or weakness of baby chicks. This finding is in agreement with Sardar and colleagues who showed that Abamectin at dose 5, 10 mg/kg causes muscle weakness and also impaired movement (Sardar *et al.*, 2020). Exposure to Abamectin insecticide results in the decrease of swimming speeds in fish after 48 hours of treatment (Kushwaha *et al.*, 2020). Moreover, insecticide affects embryos in different ways and can cause teratogenicity in chick embryos Rhode Island Red, such as unsteady gait, crooked legs and twisted phalanges (Uggini *et al.*, 2012). Other studies investigated that insecticides probably delay the development, failure of retraction yolk sac with bleeding and hematomas (Bhaskar *et al.*, 2012; Hussein and Singh, 2016). These results are in agreement with findings of the current work during treatment with Abamectin at dose 900 and 1800 ppm. From a review of previous literature, it still unknown the effect of bio insecticide on Ach E activity in the brain of chick embryo during the development. The current study, therefore, investigated that Abamectin causes significant decrease in Acetyl cholinesterase activity at 74- 97 % in the brain of chick embryo. Abass and Salih (2016) showed low activity of Ach E in rabbit's brain to 92% when they treated with Malathion insecticide.

Alhifi (2018) observed a decrease in the percentage inhibition of Ach E to 40.6% and 69% in brain tissue of chick embryo *Gallus gallus* treated with mixed insecticide (dimethoate 30% + methidathion 40%) at dose 4, 8 ppm, respectively. Pesticides interfere with physiological chemical processes and leading to morphological changes (Terali *et al.*, 2018; Uggini *et al.*, 2012; Legradi *et al.*, 2018; Hernandez *et al.*, 2020).

V. CONCLUSION

To conclude, that exposure to bio insecticide Abamectin has different effects on chicks. Treated chicks with this insecticide were physically weak with paralysis. Abamectin also inhibited the activity of acetyl cholinesterase, which gave bio indicator for pesticide poisoning. Suppressing of Ach E affected functions systems and inhibited the growth of body organs as well as abnormal change in behavior and physiological activity. Thus, Abamectin insecticide possesses physiological and morphological effects on the avian and probably on other vertebrates.

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