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An Investigation of the Toxicity of Compound Insecticide (Acetamiprid with Thiamethioxam) on the Development of Broiler Chicken Ross 308

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Abstract

Insecticides are chemical compounds that are extensively used for pest management in agriculture, urban and household environments as well as in the medicine to control different diseases such as typhus and malaria amongst others. The purpose of the current study was to investigate the toxicity effects of the compound insecticide (Acetamiprid with Thiamethioxam) on the development of Broiler chicken embryo Ross 308. Two hundred and fifty fertile eggs were divided into six groups as follows: control groups one was injected with distilled water and the other four groups were injected into the yolk sac with 100µl/egg from Acetamiprid with Thiamethioxam at concentrations (360, 540, 900 and 1800ppm). The morphological features were studied on the days 10 and 21 of incubation. The results showed that the four concentrations caused reduction in the weight of treated embryos and significant increase in the mortality. In addition, there were clear abnormalities in both ages (10 and 21) including over growth in the brain, twisted cleft beak, wry neck, eye deformation, ectopia visceral, delayed retraction of yolk sac, abnormal limbs, and broken legs. Microscopically, the liver tissue showed different changes at both incubation periods (10th day and 21st day) including necrosis and degenerative changes of hepatocytes with congestion of central vein and sinusoids with mononuclated inflammatory cells infiltration. To conclude, the current study revealed that Acetamiprid with Thiamethioxam caused significant morphological and histological changes in chicken Ross 308.

Keywords: Acetamiprid, Thiamethioxam, Insecticides, Ross 308.

الكشف عن سمية المبيد الحشري المركب (اسيتاميبرايد و ثياماثيوكسام) على تطور جنين دجاج اللحم روص 308

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الخلاصة:

مبيدات الحشرات هي مركبات كيميائية تستخدم على نطاق واسع في الزراعة لإدارة الآفات في المناطق الحضرية والمنزلية وكذلك في الطب لمكافحة امراض مختلفة مثل التيفوس والملاريا وغيرها. الغرض من هذه الدراسة الحالية هو التحقيق من التأثيرات السمية للمبيد

الحشري المركب (اسيتامبرايد و ثيامثيوكسام) على تطور جنين دجاج اللحم روص 308. استعملت في الدراسة الحالية مئتان وخمسون بيضة مخصبة قُسمت الى ستة مجاميع تضمنت مجموعة السيطرة والتي حُقن احداها بالماء المقطر, والاخرى حقنت في الكيس المحي بـ100 مايكروليتر لكل بيضة من اسيتامبرايد وثيامثيوكسام (360, 540, 900, 1800جزء بالمليون).اظهرت نتائج الدراسة الحالية وفي اليومين 10 و 21 من الحضانة انخفاض في اوزان الاجنة و زيادة كبيرة في اعداد الوفيات. بالإضافة الى ذلك, لوحظت تشوهات واضحة في كلا اليومين (10 و 21) تضمنت فرط النمو في الدماغ, تشوه واعوجاج المنقار، تشوه العين, التواء الرقبة, انتباذ الاحشاء, تأخر تراجع الكيس المحي, اطراف غير طبيعية وارجل مكسورة. كما اظهرت المقاطع النسجية للكبد تغيرات نسجية مختلفة لكلا فترتي الحضانة (10 أيام و 21 يوما) شملت التغيرات التنخر وتنكس الخلايا الكبدية واحتقان الوريد المركزي بالإضافة الى توسع الجيبانات الدموية وارتشاح الخلايا الالتهابية احادية النواة وكذلك ظهور خلايا كبفر البلعمية. يستنتج من الدراسة الحالية ان المبيد الحشري اسيتامبرايد و ثيامثيوكسام يسبب تغيرات مظهرية ونسجية كبيرة في دجاج اللحم روص 308.

الكلمات المفتاحية : أسيتامبرايد، ثيامثيوكسام، مبيدات الحشرات، روص 308.

1. Introduction

Insecticides are chemical compounds that extensively used for pest management in agriculture, urban and household environments as well as in the medicine to control different types of diseases such as typhus and malaria amongst others. These bioactive compounds are primarily designed to reduce or mitigate several species of insects by harming, repelling or killing their population. They are also highly effective in controlling soil borne and root-feeding insects [1]. Insecticides are generally classified to various divisions based on their chemistry, modes of toxic action and penetration. According to toxicity, insecticides are also commonly categorized into severity of dangerous, slightly dangerous, moderately and highly dangerous. However, insecticides are mainly classified into five groups: neonicotinoid, methyl carbamates, organophosphorus compounds, chlorinated hydrocarbons and pyrethroids insecticides [2]. Over the last decades, the agriculture sector has become increasingly interested in a class of systemic pesticides known as neonicotinoides [1]. Present neonicotinoides groups as Imidacloprid, Acetamiprid, Thiamethioxam, and Clothianidin. The compound one (Acetamiprid with Thiamethioxam) is a new neonicotinoid insecticide especially used for killing crop pests. This insecticide is effective against harmful insects that threaten agricultural products such as tobacco, tomatoes, potatoes, nuts, and cotton [2]. It has been reported that insecticides are safe materials and can be used in the environment of both animals and humans. However, long-term application of insecticides accumulates their residues in environmental components including water, food, soil and others. It, as a result, leads to adverse health impacts to animals and humans such as vomiting dizziness, nausea and headaches. Insecticide can also cause fertility problems, endocrine disorders and cancer [3], neurological disruption [4], damage the exoskeletons [5] and other serious health effects. It has been investigated that an insecticide like chlorpyrifos can interact with metronidazole antibiotic and cause acute toxicity in chicks [6]. Other studies emphasized the risk of insecticide in causing congenital abnormalities in rodents as well as avians [7,8]. The chick embryo is especially an avian model suited to investigate the embryotoxicity or teratogenicity and pharmacological study of several agents added during embryonic development. Hence, the present study aimed to investigate the teratogenicity in the chick embryo applied in ovo with Acetamiprid with Thiamethioxam mixed compound.

2. Research Method

Test Chemicals

The insecticide used in the present work was a compound from Acetamiprid with Thiamethioxam 40% WDG (Water Dispersible Granules) with commercial name Hero®, manufactured by Green River-China. The insecticide was prepared and diluted in the distilled water to four concentrations 360,540, 900 and 1800 ppm.

Procurement of eggs, injections and incubation

A total of 240 fertile broiler Ross-308 eggs were purchased from Rason company in Erbil, Iraq. The eggs were weighing (65-68g) were then cleaned by 0.5% povidone iodine to remove the external contamination. The eggs were divided into six groups and two of which were negative and positive used as controls, the positive one injected with distilled water). The eggs were injected with insecticide on the day 2. Using a plastic syringe, 100µl of insecticide was injected into the yolk sac according to the method of [9]. Control group (the positive one) received the same volume of distilled water for injection. Treated eggs were then covered by a transparent adhesive tape and incubated at 37.5 °C with a relative humidity 70-75% until embryonic days (10 or 21) period of incubation. The eggs underwent morphological observation and histopathological examination. Each fetus was separately weighed and checked for morphological abnormalities, then photographed using Nikon D5200.

Histological Examination

The liver from each chick fetus was quickly removed. According to [10], all specimens were washed in the distilled water (D.W) and then fixed in a fresh formaldehyde solution 10% for 48 hours. For microscopic observation, specimens were washed in D.W for about 2 hours followed by the subsequent processes including dehydration at room temperature. Thereafter, liver samples were embedded in the paraffin and later sectioning using a microtome. Sections were cut in 7µm thickness then processed for staining with Hematoxylin and Eosin (H&E) for examination of liver tissue. Samples were photographed by microscope digital camera SCMOS05000KPA.

Statistical Analysis

The data of the current study was analyzed by Statistical Analysis System (SAS), One way ANOVA, followed by Duncan Multiple Range. The values showed significant decrease $p < 0.05$ and $p < 0.01$.

3. Results And Discussion

Findings of ED10 incubation: Embryos mortality and weight

In the present study 120 chicken embryos on 10 ED were examined. The mortality in the treated group can be clearly observed in (Table 1). It was about 15%, 25%, 45% and 65% for doses 360, 540, 900 and 1800 ppm respectively compared to both control groups negative 0% and positive 5%. The high percentage was noted with 1800 ppm of Acetamiprid with Thiamethioxam, while the lower mortality exposed by 360. In addition, the chicken embryos treated with this insecticide showed a lower weight than the control groups (Table 1):

Table 1: Mortality percentage and weights of embryos at 10 ED after treatment with Acetamiprid with Thiamethioxam insecticide.

Experimental group	Co. Doses/egg 100µl	No. of egg treatment	No. of dead embryo	Mortality %	Weight of embryo (g)
Group A	**0**	**20**	**0**	**0**	**2.33 ± 0.14****
Group B	**100**	**20**	**0**	**0**	**2.30 ± 0.12***

Group C	**360**	**20**	**3**	**15**	**2.01 ± 0.17**
Group D	**540**	**20**	**5**	**25**	**1.27 ± 0.85***
Group E	**900**	**20**	**9**	**45**	**1.10 ± 0.20***
Group F	**1800**	**20**	**13**	**65**	**0.73 ± 0.13**

The Values represent Mean ± SE (n=10) . All values showed a significant difference from the control group at **P < 0.01, *P< 0.05.

Findings of ED10 incubation: A morphological observation of chicken embryos

Embryos in the control groups were opened at day 10th. As shown in the Figure 1 (A and B), normal embryonic development and normal organs growth for example the head, eyes, sensory organs, fore hind limbs and tail were observed in control groups. In the group injected with 360 ppm of Acetamiprid with Thiamethioxam, there was retardation in the development including over growth in the brain, twisted cleft beak and deformation of the left eye (Figure 1C) compared to the control groups. Although, there was a normal development in chick embryos treated with 540 ppm of this compound insecticide, embryos showed bending of forelimb and hind-limb (Figure 1D). In addition, there was arrested growth in the embryos treated with 900 ppm (Figure 1E) while embryos injected with 1800 ppm observed abnormal development even did not reach the desired stage (Figure 1F) in comparison with the control groups.

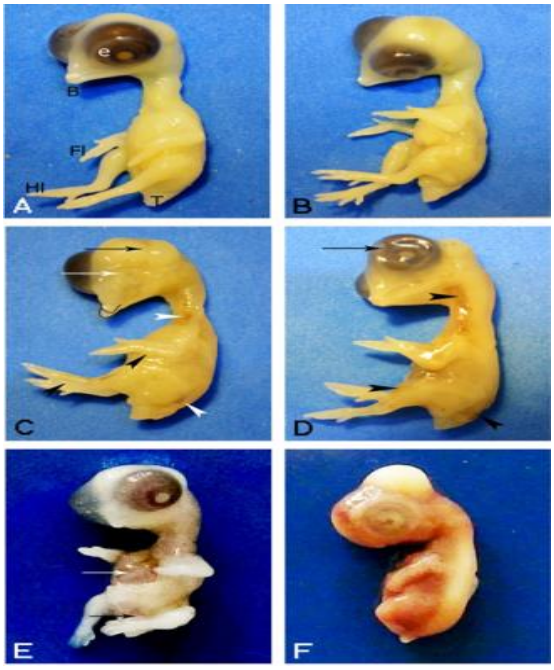

Figure 1: Shows normal embryos in the control groups A and B. C, embryos treated with 360 ppm of Acetamiprid with Thiamethioxam and can be seen abnormal embryo with deformed the left eye (white arrow), brain over growth (black arrow), twisted cleft beak and bending limbs (black head arrow) and wry neck (white hear arrow). D, embryos treated with 540 ppm of the insecticide, shows arrested growth with wry neck. E embryo injected with 900 showing arrested growth with limb deformities and ectopia viscera. F shows abnormal development in the embryo treated with 1800 ppm of Acetamiprid with Thiamethioxam. The teratogenicity parameters were observed on the chick embryos at day 10 for each dose compared with embryos in the control groups, The special features included Anophthalamus, exencephaly, beak deformities and other as shown in (Table 2).

Table 2: Shows percentages of teratogenicity parameters at 10 ED on chick embryo Ross 308

Parameters stage 10 ED	Dose 360 ppm	Dose 540 ppm	Dose 900 ppm	Dose 1800 ppm
Anophthalamus	**35**	**0**	**0**	**0**
Exencephaly	**10**	**35**	**30**	**35**
Beak deformities	**15**	**20**	**40**	**50**
Ectopia visceral	**20**	**20**	**25**	**30**
Limb deformities	**25**	**25**	**30**	**40**

All values were expressed in percentage.

Findings of ED 21 incubation: Embryos mortality and weight

On the day 21, another 120 chick embryos were used. It was shown that the mortality in the treated embryos increased significantly in chicks injected with Acetamiprid with Thiamethioxam in comparison to the control groups. The mortality was 70% in embryos injected with 360 ppm, while the higher percentage was 95% in the embryos treated with 1800 ppm compared to negative (5%) and positive (10%) control groups. Moreover, the average weight was (48g and 46g) both control groups respectively, while the injected chicken embryos with Acetamiprid with Thiamethioxam had a lower weight compared to the control groups (Table 3).

Table 3: The mortality percentage and weight of embryos at 21 ED after treatment with Acetamiprid with Thiamethioxam insecticide.

Experimental group	Doses/egg	No. of egg treatment	No. of dead embryo	Mortality %	Weight of embryo (g)
Group A	**0**	**20**	**1**	**5**	**48.3 ± 1.20***
Group B	**100**	**20**	**2**	**10**	**46.6 ± 1.45***
Group C	**360**	**20**	**13**	**70**	**40.3 ± 0.88***
Group D	**540**	**20**	**15**	**75**	**37.0 ± 2.51***
Group E	**900**	**20**	**17**	**85**	**34.3 ± 2.02***
Group F	**1800**	**20**	**19**	**95**	**31.6 ± 2.02***

The values represent Mean ±SE (n=10). All values differ significantly from the Control group at *P< 0.05.

Findings of ED 21 incubation: A morphological observation of the chicks

On the day 21 of incubation at HH stage 45, control groups chicks (positive and negative) hatched normally and were physically active. They had a proper development in the all organs including eyes, beak, limbs and claws as well as feathers (Figure 2A and B). In comparison to the control groups, treated embryos had gross malformation and morphological changes in all four doses of the insecticide of interest. In the group injected with 360 ppm of Acetamiprid with Thiamethioxam, 4 chicks out of 18 hatched normally but were not stable in walking and were physically weak. Furthermore, there was a broken leg, ectopia viscera and scanty feathers (Figure 2C). The other chicks which did not hatched normally showed arrest and abnormality in the growth, including reduction in body size and weight anophthalmia, beak defect, failure retraction in the yolk sac with crooked legs (Figure 2D). By the

exhibition of 540 ppm of the treated group, the abnormalities in embryos were higher with deformed eye, deformities in the upper beak, wry neck and congenital absence in the limbs that were shortened or bent as well as failure retraction in the yolk sac (Figure 2E). Other important signs of congenital malformations were observed in the group inoculated with 900 ppm of Acetamiprid with Thiamethioxam. In this group, eggs were un-hatched and thus help was provided. The teratogenicity included beak deformities, microphthalmia, exencephaly, crooked legs and failure retraction of yolk sac (Figure 2F). Chicks treated with 1800 ppm of Acetamiprid with Thiamethioxam showed arrested growth and high teratogenicity. Death was frequent and embryos did not reach the day 21.

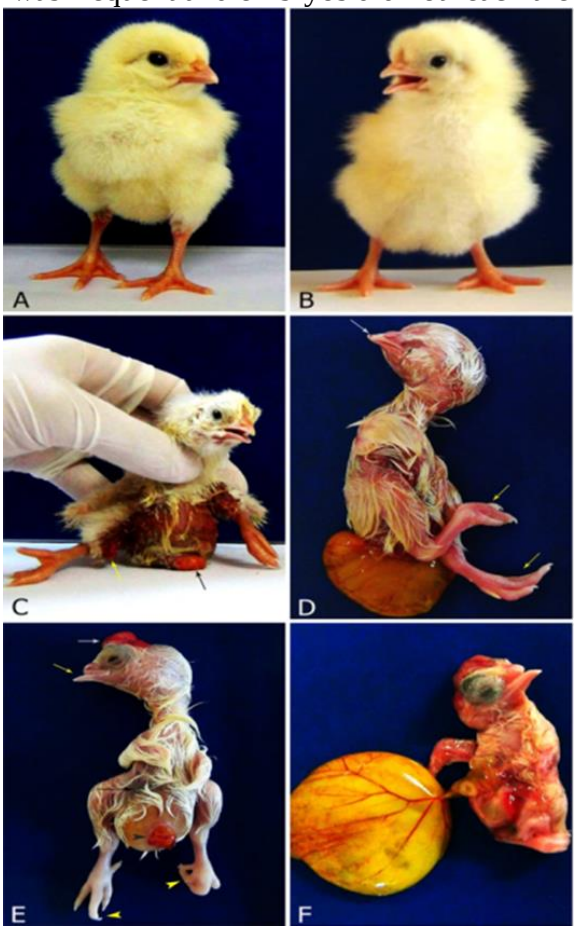

Figure 2: A and B show normal chicks in control groups. C, embryos treated with 360 ppm of Acetamiprid with Thiamethioxam can be seen as weak chicks with ectopia viscera (black arrow) broken leg (yellow arrow) and scanty feathers. D, shows an un-hatched embryo treated with 360 ppm. It shows deformed eye (black arrow), beak defect (white arrow) and failure retraction in the yolk sac with crooked legs (yellow arrows). E, embryo treated with 900 ppm of the insecticide showing defection in the beak (yellow arrow), exencephaly (white arrow), ectopia viscera (black head arrow) and crooked legs (yellow head arrows). As an embryonic development and similar to embryos on the day 10th, The teratogenic parameters were observed on the chicken embryos after 21 days of incubation and that treated with insecticides at doses 360, 540, 900 and 1800 ppm compared to the control group, the main Characteristics included Anophthalamus, Ectopia visceral, Failure retraction of yolk sac and others parameters (Table 4).

Table 4: Shows percentages of teratogenicity parameters at 21 ED on chick embryo Ross 308

Parameters stage 21 ED	Dose 360 ppm	Dose 540 ppm	Dose 900 ppm	Dose 1800 ppm
Anophthalamus	**45**	**0**	**0**	**0**
Exencephaly	**0**	**45**	**45**	**0**
Beak deformities	**30**	**45**	**40**	**0**
Ectopia visceral	**25**	**35**	**35**	**0**
Limb deformities	**35**	**35**	**45**	**60**
Wry neck	**25**	**30**	**0**	**0**
Scanty Feather	**35**	**0**	**0**	**0**
Failure retraction of yolk sac	**30**	**35**	**40**	**55**

All values were expressed at percentage.

Pesticides are classed based on their chemical composition, target and mode of action. They are used as chemical substances against a variety of pests. Insecticides are considered chemical materials used for controlling insects in agriculture, house hold practices, veterinary and medical fields. Among these Acetamiprid with Thiamethioxam are extensively used in agriculture in different forms. The current study examined the teratogenicity of this compound material insecticide on chicken Ross 308 through the development. Teratology is defined as the study of congenital abnormalities, but when an organ or organism obviously oversteps the permissible bounds of variation in any area, the condition is then called malformation or abnormality. Animal studies play an important role since they have provided insights on teratogenicity mechanisms in some cases, and because such an agent induces similar patterns of abnormalities in other species, human teratogens should also be taken into account. The current study, therefore, selected the chicken embryos as model organisms because of their genetic and structural similarities to humans, as well as their quick development period. In addition, chicks are vertebrates, easy to manipulate and control with dosing, inexpensive and also are sensitive to toxins. Studies of developmental toxicity are required to evaluate the safety of a variety of pesticides [7]. The present work investigated the teratogenicity of the compound-insecticides Acetamiprid with Thiamethioxam (neonicotinoid group), applicated on chick embryos at ED10 and ED 21 in doses (360, 540, 900 and 1800 ppm).

Over the last two decades, the agriculture sector has become increasingly interested in a class of systemic pesticides known as neonicotinoids. The present study showed that low and high doses of Acetamiprid with Thiamethioxan induced severe teratological alterations in chicken embryos. The usage of insecticide has created a chemical environment that is hazardous to living organisms. Moreover, exposure to different environmental chemicals, particularly pesticides throughout the embryonic period may be linked to the development of congenital abnormalities. Similar to the current work, it has been observed that insecticides like bendiocarb [11], bifenthrin and deltamethrin [12] as well as acetamiprid [2,7] also caused clear abnormalities in the axial and appendicular skeletal structures of chicks, rats and frogs. However, Boumezrag and colleagues found that lambda-cyhalothrin insecticide has no significant effect on the blood and mean body weight of rabbits [13]. On the other hand, poisoning with deltamethrin insecticide changes blood parameters by inhibition of

acetylcholine esterase [14]. A compound commercial insecticide Acetamiprid with Thiamethioxam which was used in the current study. It is now widely accepted that insecticide combinations can affect the non-targeted organism by contaminated water and food. Moreover, combination chemical exerts synergistic and embryo toxicity, which may result in multi-organ toxicity on consumption of contaminated food.

Once again, having knowledge that insecticides are toxicants induce teratological symptoms, the current work was designed to investigate the teratogenicity of Acetamiprid with Thiamethioxam on chicks Ross 308 during the growing period. Clothianidin, Thiamethioxam, Acetamiprid and Thiacloprid can affect the ability of mice embryo to reach the blastocyst during the development. The morphological teratogenicity observed are in line with other data working on other neonicotinoids insecticides [15, 16]. It has been found that Imidacloprid (neonicotinied insecticide) can affect the quail growth during the development [17]. Moreover, the exposure to insecticides causes defects to the neural tube and neuronal differentiation dysplasia which are effected on earliest and late stages of embryonic development [18]. Treatment of adult quail with Clothianidin, another pesticide, induces DNA fragmentation in the liver [15]. In addition, injected fertilized chicken eggs with Imidacloprid leads to obvious developmental abnormalities [16]. The embryonic cells are sensitive to neonicotinoids, which are in the order of Clothianidin < Thiomethioxam < Acetamiprid < Thiacloprid. Thiacloprid has a negative impact on quality and development of preimplantation embryos in mice and rabbits. Pandey and Mohanty discovered that co-exposure to neonicotinoid imidacloprid disrupted the hypothalamic-pituitary-thyroid (HPT) axis in the bird even at lower concentration [19]. In the last few years, it has also been noted that neonicotinoids act as endocrine disruptors and causes changes in the vertebrates' neuro-endocrine system. Several other studies also evaluated the toxicity of neonicotinoids on vertebrates, and found that Acetamiprid, Thiacloprid, Clothianidin and Thiamethioxam have a potential toxicity on mice, rabbits and chickens during the development [8, 19]. Treatment with Acetamiprid causes eye defect (anophthalmia and microphthalmia) , hypertrophy in the heart and lung hypoplasia. Acetamiprid also results in skeletal abnormalities such as a widely opened fontanel [7]. Lower concentration of Acetamiprid with Thiamethioxam affected formation of the blastocyst by decreasing cell numbers and increasing cell death [19]. Interaction between Acetamiprid with Thiamethioxam strongly increased the products toxicity, although Sarwar found that these substances partially decrease the toxicity of products [20]. The toxic impact of Acetamiprid with Thiamethioxam on chicken embryo at higher and lower doses may indicate the metabolity of these insecticides against its toxic metabolites, which are higher and lower concentrations and can be detoxified through conjugating with glutathione. Obtaining data on such inert compounds, however, is usually difficult because of the conditions of companies regarding confidential information.

Histopathology studies

Liver sections of control chick ED10 and ED21 revealed a normal hepatic architecture including hepatocytes, normal sinusoids spaces with normal central vein (Figures 3A&B and 4A&B). In a brief, histological examination of liver of chick embryos injected with 360, 540 (Figure 3C and D), 900 and 1800 ppm (Figure 3E and F) of Acetamiprid with Thiamethioxam on embryonic 10th day revealed congestion in the central vein, sinusoids dilatation with edema and congestion as well necrosis and degeneration of hepatocytes with pyknotic nuclei demonstrated inflammatory cells infiltration.

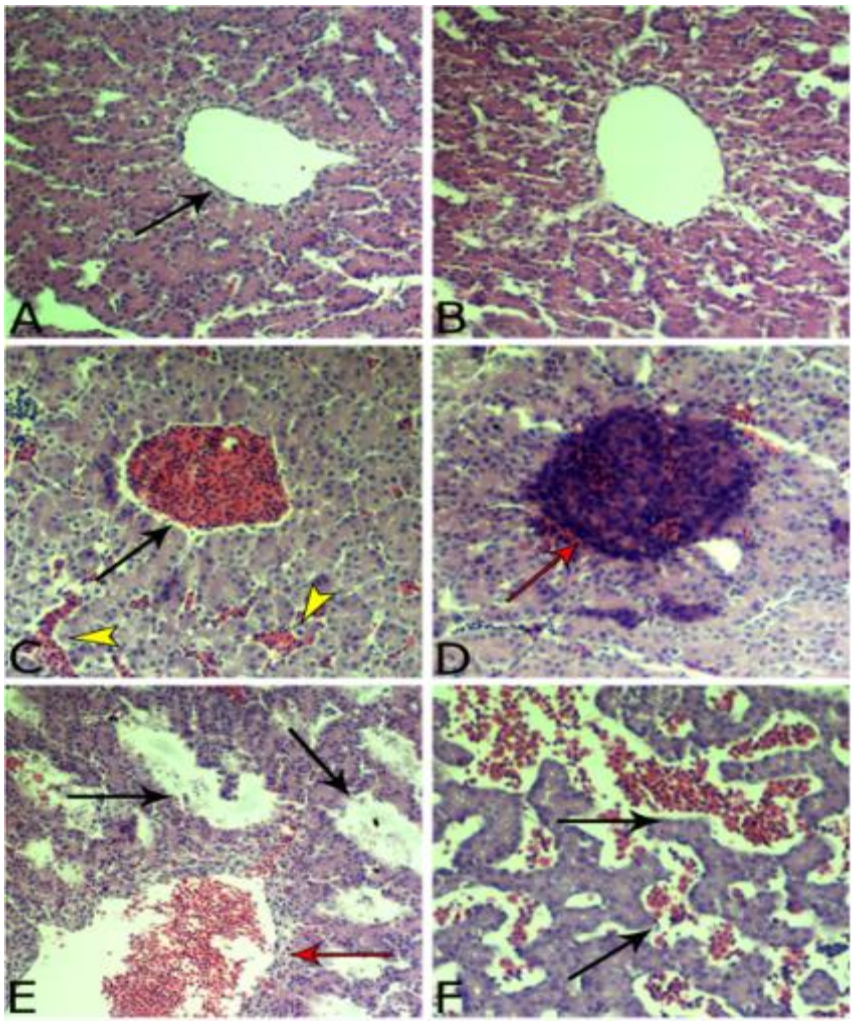

Figure 3: Photomicrographs of the da 10th chicken embryo liver sections. (A and B) section of untreated groups (negative and positive control groups respectively) showing normal radial arrangement of hepatocytes around central vein (black arrow). (C) liver section from treated embryos with 360ppm of Acetamiprid with Thiamethioxam on ED 10 shows congestion in the central vein (black arrow), swelling of sinusoids with haemorrhage (yellow head arrow). (D) section of the liver from treated with 540 ppm of the insecticide showing congestion in central vein and infiltration of inflammatory cells (red arrow). (E) section of liver from treated embryos with 900 ppm of Acetamiprid with Thiamethioxam shows swelling of sinusoids (black arrow) with congestion of central vein with vacuolization (red arrow). (F) section of liver from treated embryos with 1800 ppm of Acetamiprid with Thiamethioxam observes swelling of sinusoids (Haematoxylin and Eosin staining, magnification of 400x).

In addition, liver sections of control chicks ED 21 revealed normal hepatic architecture including hepatocytes, normal sinusoids spaces with normal central vein (Figures 4A and B). Exposure to Acetamiprid with Thiamethioxam at 360, 540 to day 21 showed marked histological alteration in the liver. As shown in the Figure 4 (C and D) there was a moderate increase in the space of sinusoids congestion in the central vein as well as necrosis and degeneration of hepatocytes. Figure (4D) also reveals cytoplasmic eosinophilia in some hepatocytes with increase of infiltration of inflammatory cells. Additionally, histological sections in the liver of 21 ED chicks treated with 900 and 1800 ppm of Acetamiprid with Thiamethioxam exhibited dilation congestion in the central vein, blood sinusoids,

fatty changes, necrosis with large vacuoles in the hepatic parenchyma and infiltration of inflammatory cells (Figure 5A and B). Embryos treated with 1800 also exhibited disturbed architecture of the hepatic cells, hepatocytes clumping, displaced nucleus, binucleation and activated Kupffer cells (Figure 5Bb). All sections were compared to control groups.

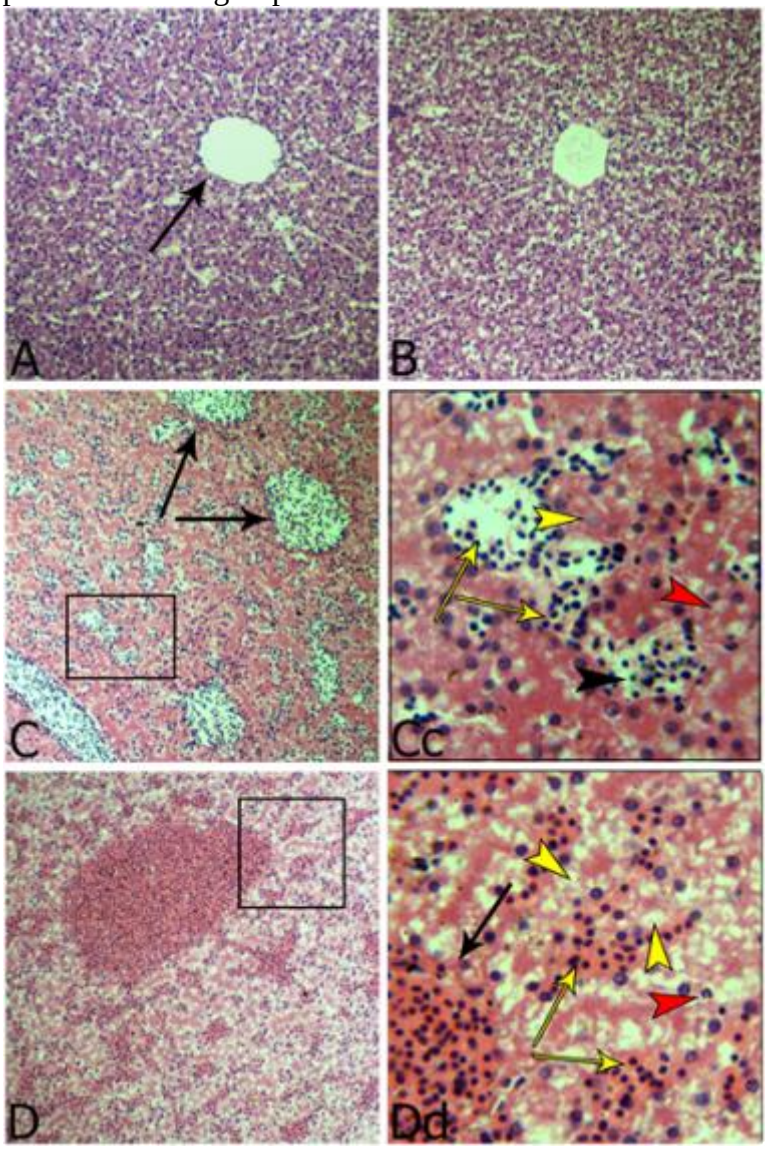

Figure 4: Photomicrographs of the 21 day old chick embryo liver sections. (A and B) sections of liver untreated groups (negative and positive control chick embryo respectively) showing normal arrangement of hepatocytes and normal of central vein (black arrow). (C and Cc, magnification 100X and 400X respectively) liver sections from treated embryos with 360 ppm of Acetamiprid with Thiamethioxam show congestion in the central vein with infiltration of inflammatory cells (black arrow), necrosis (yellow head arrow) degeneration of hepatocytes, infiltration of inflammatory cells (black head arrow), enlargement of sinusoids (yellow arrow) and vaculation (red head arrow). (D and Dd, magnification 100X and 400X respectively) the liver from treated with 540 ppm of Acetamiprid with Thiamethioxam showing loss of parenchymal and haemorrhage of the central vein (black arrow), sinusoidal dilation and hemorrhage (yellow arrows), degeneration of hepatocytes (yellow head arrows) with increase of neutrophil cells (red head arrow), (Haematoxylin and Eosin staining).

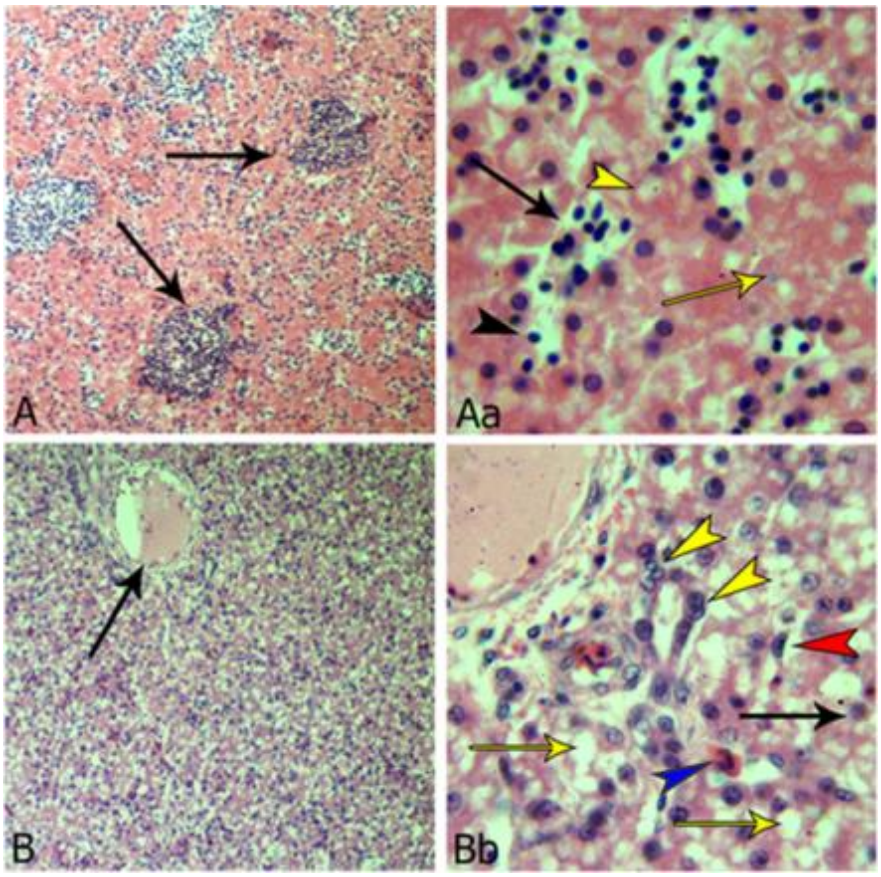

Figure 5: (A and Aa magnification is 100X and 400X respectively) liver sections from treated embryos with 900 ppm of Acetamiprid with Thiamethioxam show congestion of the central vein with severe infiltration of inflammatory cells (E, black arrow), necrosis (yellow arrow) with large vacuoles in the hepatic parenchyma (yellow head arrow), sinusoidal dilation and infiltration of inflammatory cells (black head arrow). (B and Bb, magnification 100X and 400X respectively) section of liver from treated embryos with 1800 ppm of Acetamiprid with Thiamethioxam show haemolysis of the central vein (B-black arrow), necrosis (Bb-black arrow) reticular degeneration of the hepatic cells binucleation (yellow head arrow) and activated Kupffer cells (red head arrow). Hepatocytes also observe displaced nucleus to the periphery and cytoplasmic vacuolation macrophage (Blue head arrow) (Haematoxylin and Eosin staining).

Pesticides have been shown to disrupt essential biochemical and enzymatic processes that regulate the normal lipid, glucose and protein metabolism in animals. Any change in these processes results in alteration the homeostasis and normal function of the organism. Responses to the toxicity occur frequently in the organisms including the liver, which plays a vital role in detoxification and xenobiotic metabolism. It is also considered a main organ for toxicants, which enter the portal circulatory system, once encountered or ingested [21]. In the current study, the compound insecticide (Acetamiprid with Thiamethioxam) mediated bio-chemical changes show hepatic damage in the liver section of chick embryo during the development. Herein, several histological changes shown on the liver of the chicken embryos demonstrated mild to significant cellular alterations due to the effect of different doses of this insecticide. The current work observed degeneration in the hepatocytes and necrosis. Jain and colleagues demonstrated that degeneration and necrosis in the liver cells are due to the toxicity of insecticide-

induced liver injury through hepatic oxidation mediated by cytochrome P-450 [22]. Hepatocyte necrosis could suggest oxidative stress caused by glutathione deficiency. Destruction of the membrane occurs as a result of alteration in biological properties of unsaturated lipids and proteins reside on the plasma membrane. These compounds are required for binding reactive metabolites which are ultimately responsible for unexpected changes. The histological findings of the current study were also cytoplasmic vacuolation, sinusoidal dilation, infiltrations in leucocytes and congestion as well as enlargement the central vein. Similarly, these hepatic cellular alterations have been observed on the hepatocytes of the liver of the chicken embryos exposed to dimecron (organophosphate insecticide) [23]. Vacuolated cells are due to harmful impact of insecticide on the cell membrane and degeneration of nuclear resulting in cytoplasmic vacuolation. In addition, sinusoidal dilation occurs to improve the blood flow in the hepatic lobules and then delivers nutrients and oxygen to liver cells under stress [24]. In addition, infiltration of the leucocytes may explain significant physiological tissue reactions to an insecticide-mediated toxic effect, such as hypersensitivity, inflammation and irritation.

Conclusion

According to the current study, the exposure to the insecticide, even at sub-lethal levels, has a potential toxicity on chicks. They also have toxicity on the body vital organs such as the liver. Acetamiprid with Thiamethioxam should be utilized with attention due to the dangerous effects to domestic animals as well as humans.

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6. References

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