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Exposure to Abamectin affected early embryonic development and caused changes in erythrocytes in chicken embryos (Gallus gallus)

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ABSTRACT

Early embryonic development is tightly regulated, and environmental factors can disrupt vital processes, resulting in poor embryo development. Abamectin, a pesticide and insecticide, has been shown to cause infertility in farmers. Despite its benefits, little is known about its impact on embryonic development and pregnancy. Therefore, this study aimed to examine the effects of Abamectin at two different concentrations on the nuclear erythrocyte morphology and general embryonic development in domestic Gallus chick embryos. Eggs were injected with Abamectin (ABM) at doses of 0.01 mg/kg and 0.05 mg/kg body weight per egg at two stages of development: the zero and third days of incubation. The embryos were collected on the fifth and ninth days of incubation, and their morphology was examined under a dissecting microscope. Blood samples were collected on the twelfth day of incubation. Blood smears were prepared on slides and stained; the density of blood cells, as well as the shape and abnormalities of nuclei, were examined under a light microscope.

The results from this study demonstrate that exposure to ABM led to developmental defects in embryos at stages HH29 to HH38. These defects affected overall morphology, blood vessel formation, and erythrocyte shape, with higher concentrations producing more severe outcomes.

أثر التعرض للأبامكتين على التطور الجنيني المبكر والتغيرات المستحثة في كريات الدم الحمراء في جنين الدجاج (جالس جالس)

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الكلمات المفتاحية:
أبامكتين.
التشوهات.
التطور الجنيني،
جنين الدجاج.
كريات الدم الحمراء.

الملخص

التطور الجنيني المبكر هو عملية يتم التحكم فيها بدقه حيث يمكن أن يؤدي أي اضطراب في الاستجابات الرئيسية أو مسارات الإشارة للعوامل البيئية في وقت مبكر من التطور الجنيني المبكر قد يودي الي توقف النمو الجنيني المبكر أو زيادة حالات التشوهات الجنينية.. أحد العوامل البيئية التي يجب مراعاتها هو الأبامكتين (ABM)، وهو يستخدم بشكل متكرر كمبيد حشري وعلى الرغم من تطبيقاته الإيجابية العديدة، يمكن أن يكون للأبامكتين بعض العواقب السلبية. على سبيل المثال، قد يسبب العقم لدى المزارعين الذين يستخدمونه بانتظام. على الرغم من استخدامه على نطاق واسع، لا يُعرف الكثير عن كيفية تأثيره على التطور الجنيني والحمل. لذلك، تم تصميم هذه الدراسة لمعرفة تأثير تركيز الأبامكتين على التطور الجنيني العام وشكل كريات الدم الحمراء وكذلك التغير في شكل انويه كريات الدم الحمراء في جنين كتكوت جالوس جالوس المنزلي. حيث تم حقن البيض بمادة الأبامكتين (ABM) بجرعة 0.01 ملجم/كجم و0.05 ملجم/كجم من وزن الجسم لكل بيضة على مرحلتين

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من التطور، اليوم الصفري والثالث من الحضانة، ثم تم جمع الأجنة في اليوم الخامس والتاسع من التحضين. تم فحص الشكل الخارجي للأجنة النامية تحت جهر التشريح. تم جمع عينات الدم في اليوم الثاني عشر من االتحضين تم تحضير مسحات الدم على شرائح، وتم فحص كثافة خلايا الدم وشكل النواة والتشوهات تحت المجهر الضوئي. تشير النتائج التي تم الحصول عليها من هذه الدراسة إلى أن التعرض للاباماكتين يودي الي تشوهات النمو الناجمة في المرحلتين HH29 وHH35 في الشكل العام وتكوين الأوعية الدموية وشكل كريات الدم الحمراء وتزيد حده هذه التشوهات مع زيادة التركيز.

1. Introduction

Abamectin (ABM) is a bioinsecticide widely used in agriculture to protect crops such as citrus fruits, pears, apples, potatoes, tree nuts, and various vegetables. ABM is derived from avermectin, a compound obtained from the soil bacterium Streptomyces avermitilis. It consists of two main components: the predominant avermectin B1a (80%) and the less abundant avermectin B1b (20%). Both components share a structural arrangement featuring a 16-membered ring [1,2]. ABM is neurotoxin and exhibits deworming and insecticidal effects via inhibition of the inhibitory and excitatory postsynaptic potential of neuromuscular junctions [3]. Exposure to ABM increases chloride ion concentrations, leading to polarization in nerve and muscle cells. This disruption in neuromuscular transmission ultimately causes mortality [4]. Long-term exposure to pesticides containing ABM in humans leads to the accumulation of 1.3 µg/L of ABM in the plasma [5]

Concerns are growing about the potential impacts of ABM on living organisms, as significant amounts of this substance may affect non-target organisms, including humans. While research has often focused on mammals to study ABM's pharmacokinetics, chicken embryos have also been utilized as models for drug delivery in various studies [6]. For instance, a study on zebrafish demonstrated that ABM can temporarily reduce neurotransmission activity in developing embryos, a phenomenon potentially mediated by the GABA receptor [7]. Another study assessing ABM's impact on sea urchin gametes revealed its high toxicity, as evidenced by its lytic activity on eggs and sperm, which interfered with gamete fusion and significantly reduced sperm motility [8]. The effects of ABM toxicity on liver and kidney functions have also been evaluated in male albino rats, revealing elevated levels of AST, ALT, ALP, total bilirubin, triglycerides, cholesterol, creatinine, and urea, alongside decreased albumin levels. These findings suggest adverse physiological consequences and identify potential biomarkers for monitoring ABM toxicity in mammals [9]. Similarly, studies have shown that low ABM concentrations cause severe physical abnormalities in female Coturnix japonica quail erythrocytes, with dose-dependent effects [10]. Sardar and colleagues observed that injecting ABM into fertilized eggs resulted in atypical traits, including reduced growth rates, delayed hatching, and physical weakness in chicks [11].

Moreover, ABM has been linked to infertility in farmers due to its widespread use as a pesticide and insecticide [12]. Current scientific understanding of early hematopoiesis in vertebrates is primarily derived from experiments conducted on the avian embryo. The onset of primitive hematopoiesis in the chicken embryo initiates within the blood islands of the yolk sac on the second day of incubation. During this period, the production is limited to erythrocytes and thrombocytes [13,14]. Studies on chick embryos revealed dose-dependent effects, particularly on erythrocyte morphology, emphasizing ABM's teratogenic potential in non-target organisms. It also causes physical debility, paralysis, and suppression of acetylcholinesterase, a biomarker for pesticide toxicity, resulting in physiological and morphological alterations in avian species and potentially other vertebrates [15]. A recent study further demonstrated that ABM treatment disrupted protein synthesis during embryogenesis in Argas eggs [16].

The increasing concern over the potential effects of ABM on living organisms, particularly humans, has led to research exploring the use of chicken embryos as a drug delivery mechanism [17]. The chorioallantoic membrane is utilized for efficient drug administration, overcoming the high costs, ethical concerns, and challenges of establishing and evaluating mammalian models [18]. Chicken embryos are increasingly used as a significant animal model in experimental research due to their accessibility, short embryonic development duration, genomic information accessibility, and cost-effectiveness [17]. Very recent study showed that abamectin, a common pesticide, significantly impacts zebrafish gonads, causing significant histopathological changes in ovarian and testicular tissues, including increased atretic oocytes, vacuolization, cortical alveoli hypertrophy, and structural damage [19]

2. Materials and Methods.

dissolving 0.5 g of ABM in acetone and distilled water. This solution was tested on embryos at doses of 0.01 mg/kg and 0.05 mg/kg [11]. A total of 160 fertilized Gallus gallus chicken eggs were obtained from a local agricultural facility and divided into two groups of 80 eggs each.

The first group was injected on the first day of incubation and further divided into four subgroups: a control group with no treatment, a group injected with distilled water, and two groups injected with ABM at different concentrations. Each subgroup contained 10 eggs. The second group, injected on the third day of incubation, was divided in the same manner.

Fertilized chicken eggs *Gallus gallus* were, obtained from the local farm, in the Karsa / Darna area in eastern Libya. Before incubation, eggs were sterilized by 70% ethanol alcohol. Location of blastodisc and air sac on each egg were marked on the shell using candling, were placing eggs on a light source so that it shows us a position of embryos as well as an air sac. ABM injections Before incubation, the eggs were left at room temperature and punctured at the blunt end of the egg, and about 1.5 to 2 ml of albumin was withdrawn to allow the embryo to float away from the eggshell and ABM (0.01, 0.05 mg/kg) were injected in sac with 0.60 mm diameter needle (size 23g × 1.1) , injection were created in two different stages, in zero day and in third day of incubation. After injection, the holes in the eggs were sealed with tape and the eggs were incubated at temperature 73-37.5 oc and 85% humidity to continue developing [20]

After the incubation embryos were collected at each desire stag, the eggs were removed from the incubator, the upper surface of the shell was opened with micro scissors , the embryos were transferred by spoon to Phosphate-buffered salexaminations, petri dish , and micro scissors was used them to remove excess embryonic membranes, and the embryos were examined under a dissected microscope to detect morphological defects, the embryos were photographed by digital camera (olympus) And then put in 10% formalin for further examinations , after the incubation period 12 days blood samples were took from the chicken embryos for the observations of changes in the form of red blood balls

Using a 15 cm curved tip dissection penset, the eggshell was removed, and then a 12 cm extra pointed penset was used to carefully remove the inner shell membrane. The embryo was then put on an inoculation free plate. A 15 cm curved tip dissection penset was used to swiftly remove the amniotic membrane around the embryo. After positioning the chick embryo in a supine position, sterile scissors were used to sever its sternum and ribs. In the center of the thoracic cavity was the heart, encircled by a pericardium. Using a 12-cm extra pointed penset, the pericardium was thoroughly dissected. Every chick embryo had a beating heart. Next, a 30GX13 mm mesotherapy needle was affixed to

the disposable 1 ml insulin syringe's tip to extracting blood from the heart. [21]

Five grams of agarose were dissolved in 100 ml of water and put it in a microwave. An agarose was poured into Petri dishes and left at room temperature to set, then stored until to use for photographing (Saad, 2013).Fixed embryos were photographed on the top of the agarous plate by digital camera fixed on optical technology dissected microscope. Photos of whole embryo; Head, limbs and trunk region.

Before injection, eggs were punctured at the blunt end to withdraw albumin, and ABM was injected into the air sac using a needle.

The puncture sites were sealed with tape. Eggs were incubated at 37–37.5°C and 85% humidity to allow for further development.

Embryos were collected after incubation, opened, transferred to PBS, examined under a dissecting microscope, photographed, and fixed in 10% formalin. Blood samples were taken on the twelfth day of incubation to observe changes in red blood cells. The embryos were placed on an inoculation-free plate, where the heart was dissected. Blood was extracted from the beating heart using a 30GX13 mm needle to ensure accuracy. For the blood smear preparation, the eggshell and inner membrane were carefully removed using 15 cm curved-tip dissection scissors.

Agarose Preparation: 5g of agarose was dissolved in 100ml water, microwaved, poured into Petri dishes, and set for later use in photographing.

Fixed embryos were photographed on an agarose plate using a digital camera mounted on an optical dissecting microscope. Images of the whole embryo, head, limbs, and trunk regions were captured for analysis.

3. Results

3.1. The Injection at Zero-Day:

The effects of ABM at a concentration of 0.01 mg/kg on the morphology of chicken embryos collected at developmental stages HH29 and HH35 revealed significant findings. The fertility rate was 100% across all groups, indicating good egg quality. The survival rate in the control and distilled water groups was also 100%, with no recorded deaths.

However, embryos exposed to ABM exhibited decreased survival rates, with 70% surviving at HH29 and 60% at HH35. Importantly, all surviving embryos exposed to ABM showed malformations.

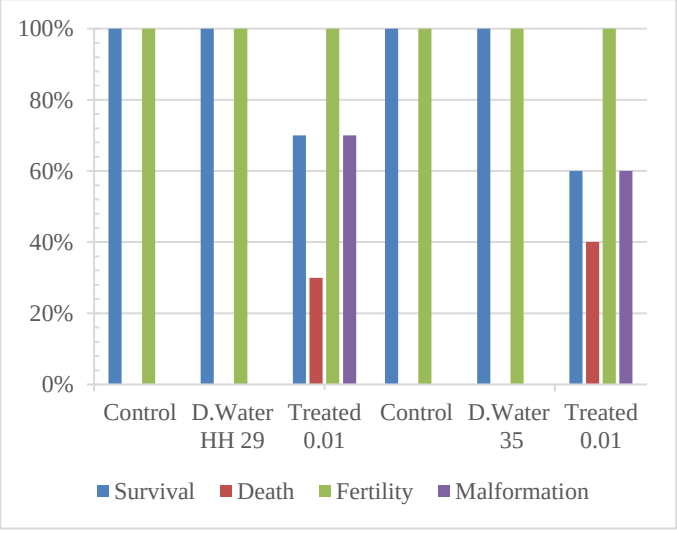

Fig. 1: The percentage of survival, death, fertility and malformation of embryos injected with 0.01 mg/kg ABM in stages HH29 and HH35.

The effect of ABM at a concentration of 0.05 mg/kg on the morphology of chicken embryos collected at HH29 and HH35:

The study found that in embryos collected at HH29 and HH35, the fertility rate was 100% in all groups, which is means egg quality was good, the survival rate was 100%, and the death rate was 0% in control and distal water groups. While ABM treatment leads to 60% in HH 29 and 100% in HH 35.

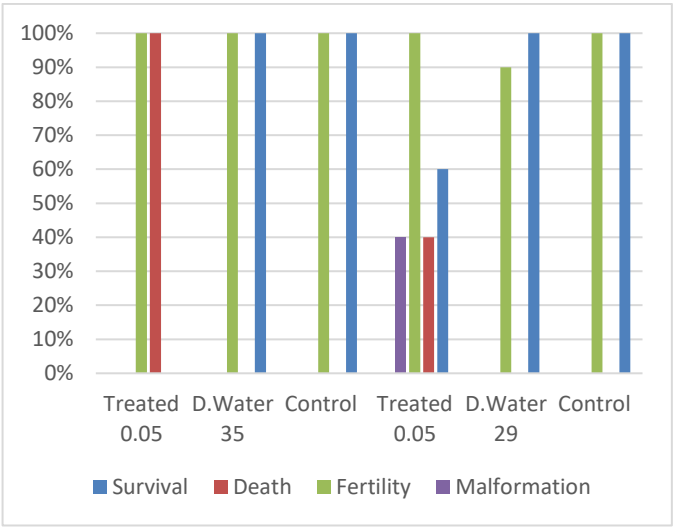

Fig. 2: The percentage of survival, death, fertility and malformation of embryos injected with 0.05 mg/kg ABM in stages HH29 and HH35.

The control group without any treatment demonstrated typical growth features, such as bent elbow wings, longer second digits, shallow grooves, knee bends, distinct toes, visible fifth toe rudiment, prominent beak, and the absence of an egg tooth, as depicted in (figure 3 A). The positive control group, which received distilled water injection, displayed similar normal growth characteristics as the previous group, as illustrated in (figure 3A). The group treated with ABM at a concentration of 0.01 mg/kg exhibited changes in the midbrain, blood accumulation in the heart red arrow in Figure 3C, and a reduction in the length of limb buds compared to the normal length of control embryos yellow arrow head figure 3C. Additionally, treatment with ABM in embryos collected at a later stage (HH 35) resulted in the death of all embryos in this group.

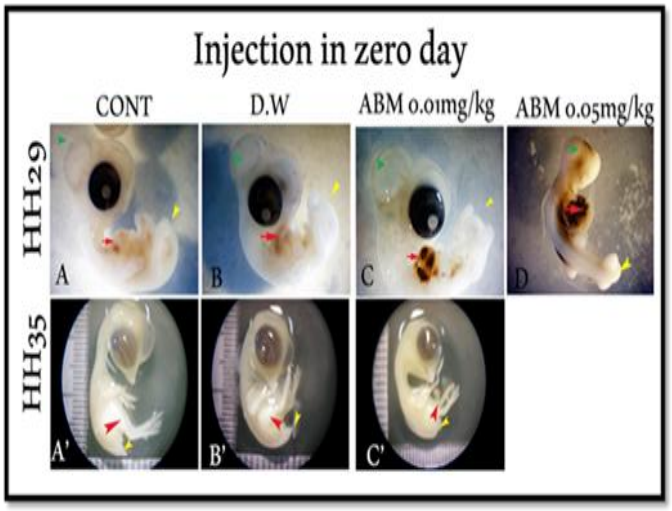

Fig 3: The effect of ABM at a concentration of 0.01 mg/kg and 0.05 mg\kg on embryo growth at different stages. Control (HH29 (A); HH35 (A'), distal water (HH29 (B); HH35 (B'), treated by abamectin (HH29 (C), HH35 (C'); red arrows point to the eye and heart.

3.2. The Injection on the Third Day

Effect of ABM at concentrations 0.01 mg/kg and 0.05 mg/kg on chicken Embryos at (HH 31 and HH35).

In this experiment, eggs were incubated for three days then injected with ABM in two concentrations 0.01 and 0.05 mg/kg, then collected at HH 31 and HH35. The results showed that the rate of fertility in all groups and treatments was 100% survival rate was 100% in control and distal water groups. While it was 80% and 70% in HH31 and HH35 respectively figure (4). The malformation rate was zero in control and distal water groups, while it was 100% in all survived embryos in both stages. Death rate was 50% and 80% in HH31 and HH 35 respectively in treatment with 0.05 mg/kg figure (5).

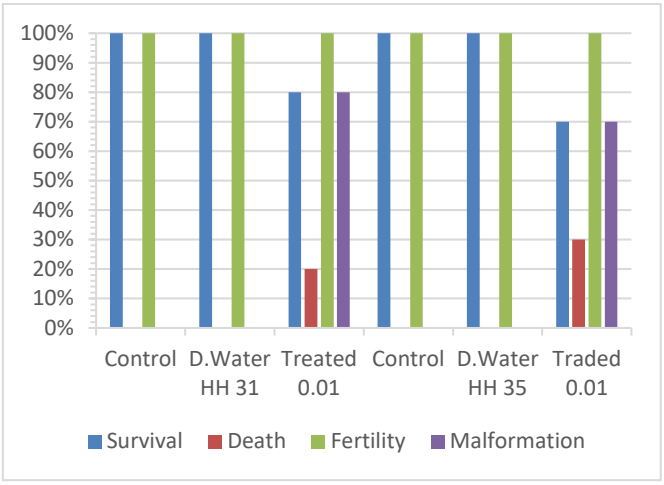

Fig. 4: The percentage of survival, death, fertility and malformation of embryos injected with 0.01 mg/kg ABM in stages HH31 and HH35

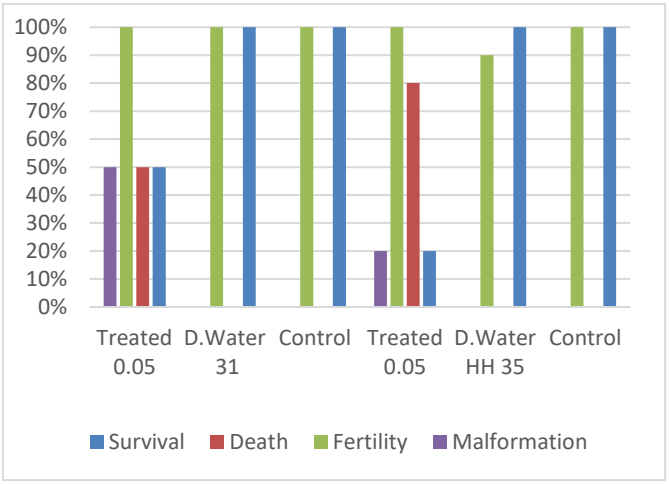

Fig. 5: Histogram showing the percentage of survival, death, fertility and malformation of embryos injected with 0.05 mg/kg ABM in stages HH31 and HH35

Injection of ABM on the third day of incubation at concentrations of 0.01 mg/kg and 0.05 mg/kg, with embryo collection on the ninth day, resulted in survival rates of 70% and 20% respectively, in comparison to 100% survival rate of the control and distilled water-treated embryos. Malformation rates were recorded at 70% and 20% for the respective concentrations, indicating that all surviving embryos exhibited malformations, as depicted in (Fig 9). Embryos treated with ABM on the third day and collected on the ninth day of incubation displayed morphological differences, such as the absence of a developed beak and reduced mass and head diameter compared to the control group.

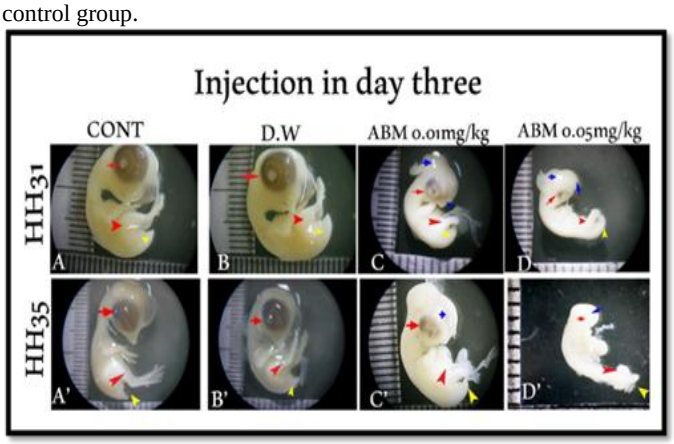

Fig 6. shows the effect of ABM at a concentration of 0.01 mg / kg and 0.05 mg\kg on embryo growth at different stages. Control (HH31 (A); HH35 (A'), distal water (HH34 (B), HH 35 (B'); treated by abamectin (HH31 (C), HH35 (C'); red arrows point to the eye and heart.

The findings of the current study indicated that both concentrations of ABM have an anti-angiogenic effect as they strongly inhibit the development of new blood vessels, and blood vessel damage was seen in treated eggs, control showed a thick network of blood vessels. The results showed that ABM caused abnormal angiogenesis, enlarged vessels and hemorrhage (Fig 7 B' and B"), and vessels atrophy (Fig 7 D' and D"), and dotted hemorrhage.

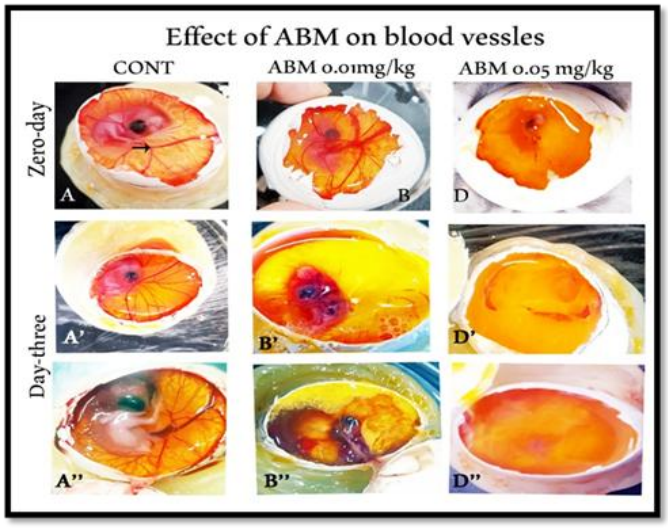

Fig 7: shows the effect of ABM at a concentration of 0.01 mg/kg and 0.05 mg/kg on the growth of blood vessels in various injections time (day zero and day three). Control (A HH29), treated with 0.01 mg/kg (B HH29) treated with 0.05 mg/kg (HH29 D) and (Control (A' and A" HH35), treated with Aba 0.01 mg\kg (B' and B" HH35), treated with Aba 0.05 mg\kg D' and D" HH35).

The blood cells staining process facilitated the differentiation between cellular components, allowing for a clearer observation of the structural characteristics of the red blood cells. Giemsa staining provided valuable insights into the cellular architecture of the blood samples. The distinct coloration of the nuclei and cytoplasm, along with the preservation of cell shape. The examination of red blood cells (RBCs) under light microscopy indicated that the control samples frequently exhibited a purple-stained nucleus, while the cytoplasm displayed a pink hue, maintaining a uniform
surface morphology. The nucleated cells retained their characteristic biconcave structure and oval configuration. The nuclei of the cells were centrally located and consistently presented an oval shape. This morphological consistency is crucial for the identification and analysis of various cell types within the blood smear as shown in figure (8 A red arrows). embryos exposed to ABM showed a higher amount of nuclear
abnormalities when compared to the control group, treated groups in figure 8 (B C, D, E and F) it showed a reduction in erythrocyte density in the field of microscope comparing with control also showed changes in cells and nuclei in shape (CSH), in fig 12 (B, C, D, E and F), binucleated erythrocytes BN in (fig 8 B). In addition to immature red blood cells (IM) in fig 8 (F), eight shaped nuclei (ESH) in fig 8 (B).

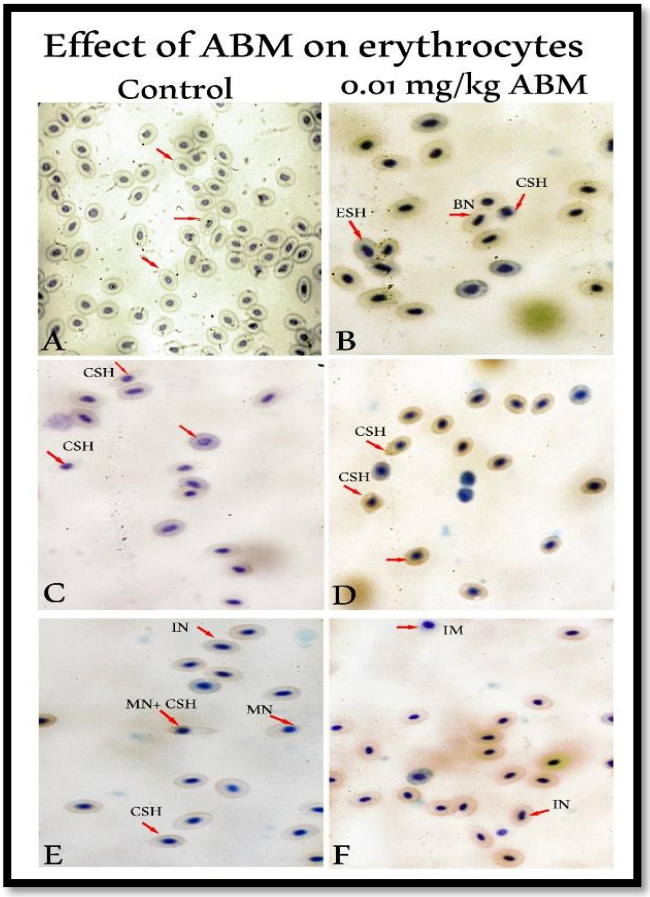

Fig 8: shows change shape erythrocytes CSH, Binucleated nucleus BN, eight shaped ESH nucleus, moved nucleus MN, indented nucleus IN, immature nucleus IM.

4.Discussion

The application of Abamectin (ABM) has increased significantly in recent years. While it offers many benefits in agricultural pest control, it also poses potential risks to non-target organisms [17]. ABM has been shown to adversely affect various animal species. Chick embryos, due to their genetic and anatomical similarities to humans and their concise developmental timeline, serve as valuable model organisms for studying toxicological effects [18].

This study aimed to examine the impact of ABM on the overall development of chick embryos and its cytotoxic effects on red blood cells. The findings demonstrated that exposure to 0.01 mg/kg of ABM caused significant developmental abnormalities, including damage to blood vessels, cardiac rupture, impaired vascular development, poor limb formation, reduced embryo size, and the absence of feather germ layers. These effects became more severe as the ABM dosage increased.

Previous research by Celik-Ozenci et al. indicated that ABM suppresses neuronal mitochondrial function and increases CASPASE-3 and CASPASE-9 activities, which are hallmarks of apoptosis. They proposed that tissue damage induced by ABM might result from PARP activation triggered by oxidative stress [22]. The lipophilic nature of ABM likely contributes to its accumulation in embryonic tissue, as observed in the present study, suggesting that direct pesticide exposure to tissues underpins its detrimental effects on embryonic development. Research conducted by Taha and Mohamed further demonstrated that ABM suppresses acetylcholinesterase activity, disrupting physiological systems and impeding organ development [15]. Similarly, the inhibition of acetylcholinesterase in the brain tissue of *Gallus gallus* embryos treated with insecticides has been associated with disrupted chemical processes essential for normal physiology, ultimately causing notable morphological alterations [23].

Hematological parameters, particularly blood cell morphology, are well-established biomarkers for assessing the environmental impacts of chemicals [24]. This study revealed that ABM exposure resulted in significant changes in erythrocyte morphology and increased irregularities in erythrocyte nuclei, highlighting ABM's cytotoxic and genotoxic potential. While ABM is widely used as a biopesticide in both veterinary and human contexts, its potential to induce genotoxicity and cytotoxicity warrants further investigation. Previous studies using various cellular models have demonstrated that even low pesticide concentrations can harm genetic material [25–27]. Future research should focus on examining the clastogenic effects of ABM through comprehensive *in vitro* and *in vivo* studies.

5. Conclusion

This study demonstrated that ABM exposure leads to significant developmental abnormalities in chick embryos, including malformations in morphological development, changes in blood cell morphology, and increased irregularities in erythrocyte nuclei. These findings underscore the cytotoxic and genotoxic effects of ABM. Although ABM is a globally popular biopesticide, its potential risks to genetic and cellular integrity remain insufficiently understood. Further research is needed to fully elucidate its toxicological profile and potential long-term impacts on non-target organisms.

6. References

[1]-Jayakumar, J. Bio-efficacy of Streptomyces avermitilis culture filtrates against root knot nematode, Meloidogyne incognita and reniform nematodes, *Rotylenchulus reniformis*. (2009). 567-571.

[2]-Takahashi, Y., Matsumoto, A., Seino, A., Ueno, J., Iwai, Y., and Omura, S. Streptomyces avermectinius sp. nov., an avermectin-producing strain. *International journal of systematic and evolutionary microbiology*, (2002). 52(6), 2163-2168.

[3]- Novick, Richard P, Christie, Gail E, and Penadés, Jose R. The phage-related chromosomal islands of Gram-positive bacteria. *Nature Reviews Microbiology*, 2010. 8(8), 541-551.

[4]-El-Saber Batiha, G., Alqahtani, A., Ilesanmi, O. B., Saati, A. A., El-Mleeh, A., Hetta, H. F., & Magdy Beshbishy, A. Avermectin derivatives, pharmacokinetics, therapeutic and toxic dosages, mechanism of action, and their biological effects. *Pharmaceuticals*, 2020. 13(8): 196.

[5]-Hunt, Julianne A, Gonzalez, Silvia, Hammond, Milton L, Pivnichny, James V, Tong, Xinchun, Xu, Suoyu S, and Guo, Qiu. 2-Arylbenzoxazoles as CETP inhibitors: Substitution and modification of the α-alkoxyamide moiety. *Bioorganic & medicinal chemistry letters*, 2010. 20(3), 1019-1022.

[6]-Darnell, D.K. and G.C. Schoenwolf. The chick embryo as a model system for analyzing mechanisms of development. *Developmental Biology Protocols*: Volume I, 2000: 25-29.

[7]-Raftery, T.D. and D.C. Volz, Abamectin induces rapid and reversible hypoactivity within early zebrafish embryos. *Neurotoxicology and Teratology*, 2015. 49: 10-18.

[8]-Tamparon, R. J. C., Bayotas, S. C., Cuadra, B. E. S., Camama, C., & Abrenica-Adamat, L. R. (2015). Dose-dependent effects of Abamectin in acute embryotoxicity and morphological defects of sea urchin (Strongylocentrotus sp.) gametes. *Aquaculture, Aquarium, Conservation & Legislation*, 2015. 8(4): 573-578.

[9]-Hamed, N. and R. Abdel-Razik, Biochemical alterations induced by abamectin in albino rats, Rattus norvegicus. *Alexandria Science Exchange Journal*, 2015. 36(JULY-SEPTEMBER): 287-273.

[10]- De Faria, D. B. G., Montalvão, M. F., de Souza, J. M., de Oliveira Mendes, B., Malafaia, G., and Rodrigues, A. S. D. L. Analysis of various effects of abamectin on erythrocyte morphology in Japanese quails (Coturnix japonica). *Environmental Science and Pollution Research*, 2018. 25(3): 2450-2456.

[11]- Sardar, K., Zafar, K., Khalid, U., Sattar, N., Mustabshira, Z., and Perveen, S. Effect of abamectin on development of chick embryo. *Pure and Applied Biology* (PAB), 2020. 9(2): 1266-1278.

[12]- Fucic, A., Duca, R. C., Galea, K. S., Maric, T., Garcia, K., Bloom, M. S., ... and Vena, J. E. Reproductive health risks associated with occupational and environmental exposure to pesticides. *International Journal of Environmental Research and Public Health*, (2021) 18(12), 6576.

[13]- Sheng, G. Primitive and definitive erythropoiesis in the yolk sac: a bird's eye view. *International Journal of Developmental Biology*. (2010) .54(6): 1033-1043.

[14]- Vink, C. S., Mariani, S. A., & Dzierzak, E. Embryonic origins of the hematopoietic system: hierarchies and heterogeneity. *Hemasphere*, 2022. 6(6), e737.

[15]- Taha, B. and R. Mohammed, Morphological, Histological Changes and Acetyl Cholinesterase Activity in Chicken Embryos After Exposure to Abamectin Insecticide. *Journal of Life and Bio Sciences Research*, 2021. 2(02):. 64-69.

[16]- Ahmed, S. S., Abotaleb, K. S., Shanbaky, N. M., Helmy, N., and Yousery, A., The effect of abamectin on some biochemical components in argas (persicargas) arboreuseggs during embryogenesis. *Egyptian Journal of Zoology*, 2024. 81(81): 61-75.

[17]- Bai, S. H., and Ogbourne, S. Eco-toxicological effects of the avermectin family with a focus on abamectin and ivermectin. *Chemosphere*, 2016. 154, 204-214.

[18]- Acharya, B., Dey, S., Sahu, P. K., Behera, A., Chowdhury, B., & Behera, S. (2024). Perspectives on chick embryo models in developmental and reproductive toxicity screening. Reproductive Toxicology, 126, 108583.

[19]- Bağdatli, S. and Yön Ertuğ, N.D., 2025. The effect of abamectin exposure on gametogenesis in zebrafish. Scientific Reports, 15(1), p.9038.19-

[20]- Acloque, H., Wilkinson, D.G. and Nieto, M.A., 2008. In situ hybridization analysis of chick embryos in whole‐mount and tissue sections. Methods in cell biology, 87, pp.169-185.

[21]- Vurmaz, A., Ertekin, A., Sabaner, M.C., Atay, E., Bozkurt, E. and Bilir, A., 2021. Effects of vitamin E in a glucocorticoid induced cataract model in chicken embryos. Biotechnic & Histochemistry, 96(6), pp.431-438.

[22]- Celik-Ozenci, C., Tasatargil, A., Tekcan, M., Sati, L., Gungor, E., Isbir, M., and Demir, R. Effects of abamectin exposure on male fertility in rats: potential role of oxidative stress-mediated poly (ADP-ribose) polymerase (PARP) activation. Regulatory Toxicology and Pharmacology, 2011. 61(3): 310-317.

[23]- Uggini, G.K., P.V. Patel, and S. Balakrishnan, Embryotoxic and teratogenic effects of pesticides in chick embryos: a comparative study using two commercial formulations. *Environmental Toxicology*, 2012. 27(3): 166-174.

[24]- Zhelev, Z., Mollova, D., and Boyadziev, P. Morphological and hematological parameters of Carassius Gibelio (Pisces: Gyprinidae) in conditions of anthropogenic pollution in Southern Bulgaria. Use of hematological parameters as biomarkers. *Trakia Journal of Sciences*, (2016). 14(1), 1-15.

[25]- Al-Sarar, A. S., Abobakr, Y., Bayoumi, A. E., and Hussein, H. I. Cytotoxic and genotoxic effects of abamectin, chlorfenapyr, and imidacloprid on CHO K1 cells. *Environmental Science and Pollution Research*, (2015). 22, 17041-17052.

[26]- Carbonell, E., Valbuena, A., Xamena, N., Creus, A., and Marcos, R. Temporary variations in chromosomal aberrations in a group of agricultural workers exposed to pesticides. *Mutation Research/Genetic Toxicology*, (1995) 344(3-4), 127-134.

[27]- Jensen, J., X. Diao, and J.J. Scott-fordsmand, Sub-lethal toxicity of the antiparasitic abamectin on earthworms and the application of neutral red retention time as a biomarker. *Chemosphere*, 2007. 68(4): p. 744-750.