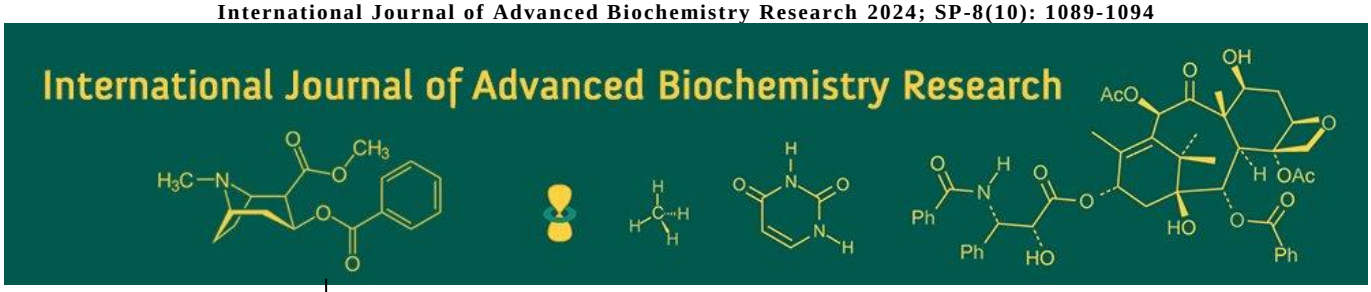

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Assessing the impact of ethion and silver nanoparticles on serum biochemical parameters in avian embryos

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Abstract
This study investigates the effects of ethion, a commonly used organophosphate pesticide, and silver nanoparticles (AgNPs) on serum biochemical parameters in developing avian embryos. Ethion treatment resulted in significant hepatotoxicity, evidenced by elevated serum levels of liver enzymes (SGOT, SGPT, ALP) and dysregulated lipid profiles, including increased total cholesterol, triglycerides, and LDL-C, with a corresponding decrease in HDL-C. In contrast, low doses of AgNPs demonstrated a protective effect, normalizing these biochemical parameters and enhancing HDL-C levels, likely due to their antioxidant properties. However, higher doses of AgNPs induced toxicity, exacerbating liver dysfunction and lipid abnormalities. The findings highlight the potential of AgNPs as a therapeutic agent to mitigate pesticide toxicity, while also underscoring the importance of dosage optimization to avoid adverse effects. This research contributes to the understanding of the interplay between pesticides and nanomaterials in avian health, advocating for further exploration into their combined effects for improved environmental and animal welfare.

Keywords: Ethion, silver nanoparticles, serum biochemisty, lipid abnormalities

1. Introduction
Pesticides are chemical substances designed to prevent, destroy, repel, or mitigate pests that can harm crops, livestock and human health (Matthews, 2015) [1]. They play a crucial role in modern agriculture by enhancing crop yield, quality, and storage (Washuck *et al*., 2022) [2]. Pesticides are broadly classified into several categories based on their target pests: insecticides (target insects), herbicides (target weeds), fungicides (target fungi), rodenticides (target rodents), and nematicides (target nematodes) (Kaur *et al*., 2019) [4]. Among these, organophosphates (OPs) represent one of the most widely used classes of insecticides due to their high efficacy in controlling a broad spectrum of pests and their relatively low persistence in the environment compared to organochlorines (Karami-Mohajeri *et al*., 2011) [4]. OPs act by inhibiting acetylcholinesterase, an enzyme essential for nervous system function, leading to the paralysis and death of pests (Pope, 1999) [5].

Ethion is an organophosphate insecticide commonly used to protect various crops, such as cotton, citrus, fruits, vegetables, and ornamentals, from pests like aphids, mites, scale insects, and leafhoppers (Dickinson, 1958) [6]. It has gained popularity due to its effectiveness in controlling these pests, ensuring better crop yields and quality (Young and Ditman, 1959) [7]. Despite its benefits in agriculture, ethion is known for its potential toxic effects on non-target species, including animals and humans (Abdel-Gawad *et al*., 2021) [8]. Ethion can induce toxicity through the inhibition of acetylcholinesterase, leading to the accumulation of acetylcholine in nerve synapses, which can result in symptoms such as muscle twitching, respiratory distress, tremors, and even paralysis in severe cases (Norma *et al*., 2022) [9]. In animals, ethion exposure has been reported to cause hepatotoxicity, nephrotoxicity, neurotoxicity, and alterations in serum biochemical parameters, reflecting its impact on organ function and metabolic processes (Krishnapur, 2020) [10]. These toxic effects highlight the need for careful management and monitoring of ethion usage in agricultural practices to minimize its environmental and health impacts (Bishnu *et al*., 2012) [11].

Silver nanoparticles (AgNPs) have garnered significant attention in recent years due to their remarkable properties and diverse applications across various fields, including medicine,

agriculture, and environmental science (Ahamed *et al*., 2010) [12]. Their unique size and high surface area-to-volume ratio contribute to their potent antimicrobial activity, making them effective against a wide range of bacteria, viruses, and fungi. This antimicrobial property is particularly valuable in agricultural practices, where AgNPs can be employed as biopesticides to control plant pathogens while minimizing the ecological footprint (Bapat *et al*., 2022) [13]. Additionally, AgNPs exhibit anti-inflammatory and antioxidant properties, enhancing their potential in therapeutic applications, such as wound healing and drug delivery systems (Paladini and Pollini, 2019) [14].

However, while silver nanoparticles offer numerous advantages, excessive use or exposure can lead to toxicity (Reidy *et al*., 2013) [15]. High concentrations of AgNPs have been associated with cytotoxic effects, including oxidative stress, inflammation, and cellular damage in various organisms. In the context of agricultural applications, overdosing with AgNPs can adversely affect non-target species, including beneficial insects and soil microorganisms, potentially disrupting ecosystems (Tlili *et al*., 2017) [16]. Furthermore, the accumulation of silver in biological tissues can lead to toxicity in higher trophic levels, including animals and humans (Xiao *et al*., 2022) [17]. Therefore, while AgNPs hold great promise for enhancing agricultural practices and improving health outcomes, careful consideration of dosage and exposure is essential to mitigate potential adverse effects and ensure their safe application (Pandian *et al*., 2015) [18].

Given this context, the present study aimed to investigate the effects of ethion, silver nanoparticles, and their combinations on developing avian embryo models to assess both ameliorative and adverse outcomes (Korani *et al*., 2015; Bhatti *et al*., 2011) [19, 20]. By exposing avian embryos to these substances, either individually or in combination, the study sought to delineate the biochemical alterations induced by ethion and evaluate whether AgNPs could counteract or exacerbate these effects. The focus was on key serum biochemical parameters, including liver enzymes, lipid profiles, and overall metabolic indicators, to provide insights into organ function and systemic health (Ozkul *et al*., 2021; Ranjbar *et al*., 2014) [22].

2. Materials and Methods

2.1 Chemicals and Reagents: Ethion was procured from Cheminova Industries Pvt. Ltd. (Mumbai, India). All other chemicals used in the study were of technical grade and obtained from reputable suppliers. Silver nanoparticles (AgNPs) were synthesized following the methodology described by Yerragopu *et al*. (2020) [23].

2.2 Egg Preparation and Handling: Fertile White Leghorn eggs were sourced from the Instructional Livestock Farm Complex (ILFC) at the College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, with study approval from the Institutional Animal Ethics Committee (IAEC/COVAS/PKD/21/01/2023 dated 12.07.2023). The eggs were disinfected with 70% alcohol, dried, weighed to approximately 50±5 grams, and labeled. They were candled to discard any defective eggs and mark the air cell position, then incubated at 37.8°C with 55% humidity, turning them every four hours (Adame and Ameha, 2023) [24]

2.3 Inoculation via Yolk Sac: On the fifth day of incubation, 120 eggs were candled to observe the vitelline vessels. Each egg was subsequently inoculated with a 100 µL dose containing ethion, silver nanoparticles (AgNPs), or their combinations via the yolk sac (Bhanja *et al*., 2004) [25]. The eggs were allocated into ten groups: vehicle control, silver nanoparticle group, ethion treatment groups, and their combinations. The specifics of the experimental design are outlined in the table below.

2.4 Experimental Grouping and Design: In this study, 10 groups of 12 embryonated eggs each were utilized to evaluate the effects of silver nanoparticles and ethion, both individually and in combination, on avian embryo development. Group 1 served as the control, receiving 100 µL of olive oil. Groups 2, 3, and 4 received silver nanoparticles at doses of 25, 50, and 100 µg/egg, respectively, while Groups 5, 6, and 7 were treated with ethion at doses of 73.5, 147, and 294 µg/egg, all in 100 µL of olive oil. Groups 8, 9, and 10 received combined treatments of AgNP-25 µg/egg + ETH-73.5 µg/egg, AgNP-50 µg/egg + ETH-147 µg/egg, and AgNP-100 µg/egg + ETH-294 µg/egg, respectively. Dosing was initiated on the 5th day of incubation, and the eggs were incubated until day 19, when they were opened for biochemical assessment to evaluate the effects of the treatments, as detailed in Table 1.

Table 1: Treatment and dosing schedule for *In-ovo* experimentation with ethion, AgNPs, and their combinations in the developing avian embryo model:

Groups	Treatment	Dose/ Route (*In-ovo*)	No. of eggs
1	Vehicle	Olive oil @ 100 µL/egg	12
2	Silver Nanoparticle (AgNP-25)	25 µg/egg in 100 µL of olive oil	12
3	Silver Nanoparticle (AgNP-50)	50 µg/egg in 100 µL of olive oil	12
4	Silver Nanoparticle (AgNP-100)	100 µg/egg in 100 µL of olive oil	12
5	Ethion (ETH-73.5)	73.5 µg/egg in 100 µL of olive oil	
6	Ethion (ETH-147)	147 µg/egg in 100 µL of olive oil	12
7	Ethion (ETH-294)	294 µg/egg in 100 µL of olive oil	
8	Silver Nanoparticle + (AgNP-25) Ethion (ETH-73.5)	Silver nanoparticle (25 µg/egg) in 100 µL of olive oil+ Ethion (73.5 µg/egg) in 100 µL of olive oil	12
9	Silver Nanoparticle (AgNP-50) +Ethion (ETH-147)	Silver nanoparticle (50 µg/egg) in 100 µL of olive oil +Ethion (147 µg/egg) in 100 µL of olive oil	12
10	Silver Nanoparticle (AgNP-100) + Ethion (ETH-294)	Silver nanoparticle (100 µg/egg) in 100 µL of olive oil + Ethion (294 µg/egg) in 100 µL of olive oil	12

2.5 Serum Biochemical Assessment: On the 19th day of incubation, blood samples were collected for serum biochemical analysis. Approximately 1 mL of blood was extracted from each embryo via jugular puncture into serum

separator tubes. The samples were analyzed for serum biochemical parameters, including Total protein, albumin, SGOT, SGPT, alkaline phosphatase (ALP), triglycerides, total cholesterol, LDL-C, and HDL-C using Corel Clinical diagnostics kit as per manufacturer instructions.

2.6 Statistical Analysis: The data are presented as Mean ± SEM (n=12). Statistical significance was determined using one-way analysis of variance, followed by Tukey's multiple comparison test, conducted with SPSS Version 24.0.

3. Results and Discussion

Table 2: Effect of *In-ovo* administration of Ethion (ETH), Silver nanoparticles (AgNp) and their combination on serum biochemical parameters on the 19th day of avian embryo development.

Treatment	Total Protein (g/dl)	Albumin (g/dl)	SGOT (U/L)	SGPT (U/L)	Glucose (mg/dl)	ALP (U/L)
Vehicle	4.658±0.18^{c}	1.756±0.06^{c}	163.25±2.89^{a}	24.69±1.02^{a}	228.36±2.68^{c}	269.38±3.08^{a}
AgNP-25 (A1)	4.721±0.17^{c}	1.782±0.05^{c}	165.35±2.59^{a}	24.58±1.21^{a}	229.68±2.57^{c}	271.36±3.09^{a}
AgNP-50 (A2)	4.692±0.16$_{c}$	1.732±0.06^{c}	172.52±2.57^{a}	25.62±1.09^{a}	231.52±2.39^{c}	268.35±3.02^{a}
AgNP-100 (A3)	3.627±0.19^{b}	1.426±0.07^{b}	188.35±2.64^{b}	32.32±1.12^{b}	201.64±2.54^{b}	325.27±2.98^{b}
ETH-73.5 (E1)	4.698±0.17^{c}	1.786±0.05^{c}	167.24±2.53^{a}	25.68±1.19^{a}	227.63±2.62^{c}	268.32±2.96^{c}
ETH-147 (E2)	3.658±0.16^{b}	1.421±0.06^{b}	208.35±2.51^{c}	36.57±1.09^{c}	186.47±2.57^{b}	328.15±2.84^{c}
ETH-294 (E3)	3.128±0.18^{a}	1.321±0.08^{a}	221.02±2.46^{d}	41.86±1.21^{d}	174.68±2.36^{a}	346.27±3.07^{d}
AgNP-25 +ETH-73.5 (AE1)	4.657±0.19^{c}	1.786±0.05^{c}	168.35±2.38^{a}	28.69±1.09^{a}	228.67±2.57^{c}	271.08±3.06^{a}
AgNP-50 +ETH-147 (AE2)	4.562±0.18^{c}	1.749±0.04^{c}	168.25±2.49^{a}	26.38±1.21^{a}	229.62±2.49^{c}	270.58±2.85^{a}
AgNP-100+ ETH-294 (AE3)	3.258±0.19^{a}	1.325±0.06^{a}	220.35±2.56^{d}	42.64±1.27^{d}	176.14±2.63^{a}	347.38±2.96^{d}

Units are expressed as grams (g), milligram (mg), dL (decilitre), U (units), L (litre), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP). Values are expressed as Mean ± SEM (n=12). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. comparison was made between each groups having different superscripts denoting the level of significance indicates $p<0.05$ respectively.

Serum biochemical parameters, including total protein, albumin, SGOT, SGPT, glucose and ALP were evaluated in avian embryos on the 19th day of development. These embryos were treated with ethion, silver nanoparticles, or combinations thereof. Results revealed a significant ($p < 0.05$) decrease in total protein, albumin and glucose values in groups A3, E2, E3, and AE3 compared to the control. Conversely, groups AE1 and AE2 demonstrated a significant ($p < 0.05$) improvement in these parameters, approaching levels comparable to the control group. The values of SGOT, SGPT and ALP shown an significant increase in groups A3, E2, E3 and AE3 compared to control. However, groups AE1 and AE2 showed a significant ($p<0.05$) improvement comparable with the control group.

Table 3: Effect of *In-ovo* administration of Ethion (ETH), Silver nanoparticles (AgNp) and their combination on total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterols (HDL-C) parameters on the 19th day of avian embryo development.

Treatment	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Vehicle	365.42±2.58^{a}	119.63±1.95^{a}	68.66±1.09^{a}	46.69±1.28^{c}
AgNP-25 (A1)	358.35±2.65^{a}	118.67±1.98^{a}	68.39±1.05^{a}	47.85±1.06^{c}
AgNP-50 (A2)	359.24±2.49^{a}	120.02±1.96^{a}	69.84±0.95^{a}	49.02±1.27^{c}
AgNP-100 (A3)	402.58±2.52^{b}	135.58±2.08^{b}	75.89±0.89^{b}	40.32±1.38^{b}
ETH-73.5 (E1)	360.24±2.56^{a}	120.57±2.04^{a}	68.58±0.84^{a}	47.98±1.34^{c}
ETH-147 (E2)	432.35±2.62^{c}	141.36±2.06^{c}	81.25±1.08^{c}	39.96±1.34^{b}
ETH-294 (E3)	457.09±2.58^{d}	149.54±2.08^{d}	87.95±0.87^{d}	30.52±1.29^{a}
AgNP-25 +ETH-73.5 (AE1)	361.58±2.62^{a}	120.47±1.95^{a}	69.24±0.92^{a}	47.02±1.27^{c}
AgNP-50 +ETH-147 (AE2)	357.25±2.59^{a}	119.68±1.96^{a}	67.98±1.05^{a}	46.85±1.26^{c}
AgNP-100+ ETH-294 (AE3)	458.01±2.64^{d}	148.58±1.94^{d}	86.02±1.07^{d}	29.29±1.27^{a}

Units are expressed as grams(g), milligram (mg), decilitre (dl), low density lipoprotein-cholestrol (LDL-C), high density lipoprotein cholesterol (HDL-C). Values are expressed as Mean ± SEM (n=12). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. comparison was made between each groups having different superscripts denoting the level of significance indicates $p<0.05$ respectively.

The administration of ethion, silver nanoparticles, or their combinations to avian embryos on the 19th day of development significantly decreased HDL-C levels in groups A3, E2, E3 and AE3 respectively as compared to the control groups. However the groups AE1 and AE2 showed an significant ($p<0.05$) improvement in the levels of HDL-C and are comparable with control groups. The values of triglycerides, total cholesterol and LDL-C showed an significant ($p<0.05$) increased in A3, E2 and AE3 as compared to the control group. However the groups AE1 and AE2 showed an amelioration and are comparable with the control group.

Serum biochemistry plays a crucial role in understanding the physiological and metabolic changes that occur in response to toxic substances. It provides valuable insights into the overall health and functioning of vital organs, such as the liver, kidneys, and cardiovascular system (Wolf, 1999) [26]. By analyzing various biochemical markers, we can detect early signs of toxicity, metabolic disturbances, or organ damage (Ramaiah, 2007) [27]. In this study, the evaluation of serum biochemistry allowed us to comprehensively assess the impact of ethion and silver

nanoparticles on avian embryos, highlighting their potential toxic effects and the body's response to these exposures. This makes serum biochemistry an indispensable tool for toxicity assessment and understanding the effects of toxicants in biological systems (Singh *et al*., 2011) [28].

In the ethion-treated group of avian embryos, the observed reduction in total protein, albumin, and glucose levels can be attributed to ethion's hepatotoxic effects (Durom *et al*., 2024) [29]. The liver is responsible for producing albumin, a key protein in the blood that helps maintain osmotic pressure and transport various substances. Ethion disrupts liver function, leading to impaired protein synthesis and gluconeogenesis, which are critical for maintaining normal levels of these parameters (Singh *et al*., 2006) [30]. Ethion's toxicity may also affect glucose metabolism, contributing to decreased serum glucose levels (Kadar e al., 2017) [31].

In the highest dose group of silver nanoparticles (AgNPs), the decrease in total protein, albumin, and glucose levels may indicate a dose-dependent toxicity (Tiwari *et al*., 2011) [32]. High concentrations of AgNPs can induce oxidative stress and inflammation, adversely affecting liver function and leading to similar impairments as seen with ethion (Wen *et al*., 2022) [33]. This toxicity could hinder the liver's ability to synthesize proteins and regulate glucose levels, exacerbating the effects of ethion (Bhatti *et al*., 2011) [34].

Conversely, the 1/10 dose of silver nanoparticles pre-treated with ethion exhibited an ameliorative effect, potentially due to the nanoparticles' antioxidant properties and their ability to modulate inflammatory responses (Priya *et al*., 2016) [35]. At lower concentrations, AgNPs may enhance the liver's ability to recover from ethion-induced damage, promoting protein synthesis and normalizing glucose levels (Reshi *et al*., 2017; Elfaky *et al*., 2022) [36-37]. However, when the dosage of silver nanoparticles is increased beyond a certain threshold, the adverse effects can outweigh the benefits, leading to toxicity, as observed in the highest treatment group (Rana, 2020) [38]. The dual nature of AgNPs are beneficial at low doses and harmful at high doses that underscores the importance of dosage optimization in therapeutic applications (Kaushal *et al*., 2023) [39].

In the ethion-treated group of avian embryos, the increased levels of SGOT (AST), SGPT (ALT), and ALP are indicative of liver damage and cellular leakage (Ranjith *et al*., 2022) [40]. Ethion, being an organophosphate, can induce hepatotoxicity by disrupting normal liver function and causing hepatocyte injury (Shukla e al., 2021) [41]. This leads to the release of these enzymes into the bloodstream, reflecting the extent of liver damage (Bhatti *et al*., 2014) [42]. In the highest dose group of silver nanoparticles (AgNPs), the elevated levels of SGOT, SGPT, and ALP may suggest a similar hepatotoxic effect (Kaushik and Joshi, 2016) [43]. At high concentrations, AgNPs can generate oxidative stress, triggering inflammation and cellular damage in the liver, thus leading to increased enzyme levels (Kim *et al*., 2009) [44]. In contrast, the 1/10 dose of silver nanoparticles pre-treated with ethion demonstrated ameliorative potential, likely due to AgNPs' antioxidant properties, which may help to mitigate oxidative stress and protect liver cells from further damage (Priya *et al*.,2016) [45]. This protective effect could facilitate the maintenance of normal liver function and reduce enzyme leakage, thus normalizing SGOT, SGPT, and ALP levels (Reshi *et al*., 2017; Elfaky *et al*., 2022) [36, 37].

However, when the dose of silver nanoparticles is too high, the toxic effects can dominate (Piao *et al*., 2011) [47]. High concentrations of AgNPs may overwhelm the liver's capacity to cope with oxidative stress and inflammation, leading to increased levels of these enzymes due to exacerbated liver damage, similar to or even worse than that induced by ethion alone (Bhatti *et al*., 2014) [42]. This highlights the importance of carefully managing dosages to harness the therapeutic benefits of AgNPs while avoiding potential toxicity (Kaushal *et al*., 2023) [39]. In the ethion-treated group of avian embryos, the increased levels of total cholesterol (TC), triglycerides (TG), and LDL-C, along with decreased HDL-C, can be attributed to ethion's hepatotoxic effects (Bhatti *et al*., 2011) [50]. Ethion disrupts liver function, impairing the metabolism of lipids. The liver plays a crucial role in lipid regulation, and its damage can lead to an accumulation of cholesterol and triglycerides in the bloodstream, while simultaneously reducing the synthesis of HDL-C, which is responsible for transporting cholesterol away from the arteries (Shukla *et al*., 2022) [46]. In the highest dose group of silver nanoparticles (AgNPs), the observed increase in TC, TG, and LDL-C, along with decreased HDL-C, may reflect a similar pattern of liver stress and lipid dysregulation (Sulaiman *et al*., 2015) [47]. High concentrations of AgNPs can induce oxidative stress and inflammation, negatively affecting liver function and leading to lipid profile alterations akin to those caused by ethion (Elle *et al*., 2013) [48].

Conversely, the 1/10 dose of silver nanoparticles pre-treated with ethion showed ameliorative potential by normalizing the increased levels of TC, TG, and LDL-C while increasing HDL-C levels (Priya *et al*., 2016). This protective effect may stem from the antioxidant properties of AgNPs at lower concentrations, which can help reduce oxidative stress, improve liver function, and enhance the synthesis of HDL-C, thereby restoring a healthier lipid profile (Elfaky *et al*., 2022) [37]. However, at high doses, silver nanoparticles can exhibit toxicity, potentially leading to further liver damage and exacerbated lipid profile disturbances (El Mahdy *et al*.,2015) [49]. The toxicity may arise from overwhelming oxidative stress, inflammation, or direct cellular injury, thereby negating any protective effects and resulting in worsened lipid abnormalities similar to those seen with high doses of ethion (Kaushik and Joshi, 2016) [43].

In summary, the study examined the effects of ethion, silver nanoparticles (AgNPs), and their combinations on developing avian embryos, revealed that ethion caused significant hepatotoxicity and disrupted lipid metabolism, evidenced by increased liver enzyme levels, elevated total cholesterol, triglycerides, and LDL-C, alongside decreased HDL-C (Korani *et al*., 2015; Bhatti *et al*., 2011) [19, 20]. Silver nanoparticles demonstrated a protective effect at low doses, normalizing these parameters and enhancing HDL-C levels, likely due to their antioxidant properties (Reshi *et al*., 2017) [36]. However, high doses of AgNPs induced toxicity, worsening liver function and lipid profiles similarly to ethion. This underscores the potential of AgNPs to mitigate pesticide toxicity while highlighting the risks of excessive dosage (Kaushal *et al*., 2023) [39].

4. Conclusion

In conclusion, this study highlights the significant impact of ethion on serum biochemical parameters in developing avian embryos, demonstrating its hepatotoxic effects through increased liver enzymes and altered lipid profiles.

The protective role of silver nanoparticles (AgNPs) at lower doses suggests their potential as a mitigating agent against pesticide-induced toxicity, promoting normalization of lipid levels and enhancing overall liver function. However, the observed toxicity at higher doses of AgNPs indicates a complex dose-response relationship that warrants careful consideration in therapeutic applications. Overall, these findings emphasize the need for further research to explore the safe and effective use of silver nanoparticles in mitigating the adverse effects of agricultural pesticides, ultimately contributing to improved animal health and environmental safety.

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6. Conflict of Interest

The authors declare that there is no conflict of interest in this work. All research activities were conducted with complete academic integrity and transparency, and there are no financial, personal, or professional relationships that could have influenced the outcome or interpretation of this study.

7. References

1. Matthews G. Pesticides: health, safety and the environment. John Wiley & Sons; c2015 Oct 26.
2. Washuck N, Hanson M, Prosser R. Yield to the data: some perspective on crop productivity and pesticides. Pest Management Science. 2022 May;78(5):1765-71.
3. Kaur R, Mavi GK, Raghav S, Khan I. Pesticides classification and its impact on environment. Int. J. Curr. Microbiol. Appl. Sci. 2019 Mar 20;8(3):1889-97.
4. Karami-Mohajeri S, Abdollahi M. Toxic influence of organophosphate, carbamate, and organochlorine pesticides on cellular metabolism of lipids, proteins, and carbohydrates: a systematic review. Human & experimental toxicology. 2011 Sep;30(9):1119-40.
5. Pope CN. Organophosphorus pesticides: do they all have the same mechanism of toxicity?. Journal of Toxicology and Environmental Health Part B: Critical Reviews. 1999 Mar 1;2(2):161-81.
6. Dickinson BC. Ethion, a promising new acaricide and insecticide. Journal of Economic Entomology. 1958 Jun 1;51(3):354-57
7. Young JR, Ditman LP. The effectiveness of some insecticides on several vegetable crops. Journal of Economic Entomology. 1959 Jun 1;52(3):477-81.
8. Abdel-Gawad H, Soliman S, Taha H, Aly M, A Abd El Kader M. Pathogenic effects of Ethion residues and the expected protective role of the Ethanolic extract of rosemary (*Rosmarinus officinalis* L.) leaves in male rats. Egyptian Journal of Chemistry. 2021 Apr 1;64(4):1817-29.
9. Norma PG, Shathani M, Thato P, Tsenang M. Multiple causalities due to ethion pesticide poisoning: A case report. Am J Biomed Sci Res. 2022 Feb 11;15(3):352-56.
10. Krishnapur SY. Eradication of Histopathological and biochemical changes in testes and reproductive organs on Exposure to organophosphate, ethion. Technium BioChemMed. 2020 Dec 10;1(1):1-0.
11. Bishnu A, Chakraborty A, Chakrabarti K, Saha T. Ethion degradation and its correlation with microbial and biochemical parameters of tea soils. Biology and Fertility of Soils. 2012 Jan;48:19-29.
12. Ahamed M, AlSalhi MS, Siddiqui MK. Silver nanoparticle applications and human health. Clinica chimica acta. 2010 Dec 14;411(23-24):1841-8.
13. Bapat MS, Singh H, Shukla SK, Singh PP, Vo DV, Yadav A, Goyal A, Sharma A, Kumar D. Evaluating green silver nanoparticles as prospective biopesticides: An environmental standpoint. Chemosphere. 2022 Jan 1;286:131761.
14. Paladini F, Pollini M. Antimicrobial silver nanoparticles for wound healing application: progress and future trends. Materials. 2019 Aug 9;12(16):2540.
15. Reidy B, Haase A, Luch A, Dawson KA, Lynch I. Mechanisms of silver nanoparticle release, transformation and toxicity: A critical review of current knowledge and recommendations for future studies and applications. Materials. 2013 Jun;6(6):2295-350.
16. Tlili A, Jabiol J, Behra R, Gil-Allué C, Gessner MO. Chronic exposure effects of silver nanoparticles on stream microbial decomposer communities and ecosystem functions. Environmental science and technology. 2017 Feb 21;51(4):2447-55.
17. Xiao B, Yang R, Chen P, Yang J, Sun B, Wang K. Insights into the lower trophic transfer of silver ions than silver containing nanoparticles along an aquatic food chain. Science of The Total Environment. 2022 Jan 15;804:150228.
18. Pandian AM, Karthikeyan C, Rajasimman M, Dinesh MG. Synthesis of silver nanoparticle and its application. Ecotoxicology and environmental safety. 2015 Nov 1;121:211-7.
19. Korani M, Ghazizadeh E, Korani S, Hami Z, Mohammadi-Bardbori A. Effects of silver nanoparticles on human health. European Journal of Nanomedicine. 2015 Jan 1;7(1):51-62.
20. Bhatti GK, Bhatti JS, Kiran R, Sandhir R. Biochemical and morphological perturbations in rat erythrocytes exposed to ethion: protective effect of vitamin E. Cellular and Molecular Biology. 2011 Feb 12;57(1):70-9.
21. Özkul T, Günal SY, Aydoğan S. The effect of ethion on erythrocyte deformability. Pesticide biochemistry and physiology. 2021 Feb 1;172:104753.
22. Ranjbar A, Ataie Z, Khajavi F, Ghasemi H. Effects of silver nanoparticle (Ag NP) on oxidative stress biomarkers in rat. Nanomed J. 2014 Apr 1;1(3):205-10.
23. Yerragopu PS, Hiregoudar S, Nidoni U, Ramappa KT, Sreenivas AG, Doddagoudar SR. Chemical synthesis of silver nanoparticles using tri-sodium citrate, stability study and their characterization. International Research Journal of Pure and Applied Chemistry. 2020 Mar 24;21(3):37-50.
24. Adame MM, Ameha N. Review on Egg Handling and Management of Incubation and Hatchery Environment. Asian J. Biol. Sci. 2023;16(4):474-84.
25. Bhanja SK, Mandal AB, Johri TS. Standardization of injection site, needle length, embryonic age and

concentration of amino acids for in ovo injection in broiler breeder eggs. Indian Journal of Poultry Science. 2004;39(2):105-11.
26. Wolf PL. Biochemical diagnosis of liver disease. Indian Journal of Clinical Biochemistry. 1999 Jan;14:59-90.
27. Ramaiah SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food and chemical toxicology. 2007 Sep 1;45(9):1551-7.
28. Singh A, Bhat TK, Sharma OP. Clinical biochemistry of hepatotoxicity. J Clinic Toxicol S. 2011;4:2161-0495.
29. Durom EG, Aneesha VA, Kumar NV, Azeez AB, Karikalan M, Lingaraju MC. Prenatal exposure to ethion caused maternal and foetal toxicity in rats. Reproductive Toxicology. 2024 Jun 1;126:108607.
30. Singh M, Sandhir R, Kiran R. Erythrocyte antioxidant enzymes in toxicological evaluation of commonly used organophosphate pesticides. Indian Journal of Experimental Biology. 2006 Jul 1;44(7):580.
31. Kadar A, De Sousa G, Peyre L, Wortham H, Doumenq P, Rahmani R. Evidence of in vitro metabolic interaction effects of a chlorfenvinphos, ethion and linuron mixture on human hepatic detoxification rates. Chemosphere. 2017 Aug 1;181:666-74.
32. Tiwari DK, Jin T, Behari J. Dose-dependent in-vivo toxicity assessment of silver nanoparticle in Wistar rats. Toxicology mechanisms and methods. 2011 Jan 1;21(1):13-24.
33. Wen L, Li M, Lin X, Li Y, Song H, Chen H. AgNPs aggravated hepatic steatosis, inflammation, oxidative stress, and epigenetic changes in mice with NAFLD induced by HFD. Frontiers in Bioengineering and Biotechnology. 2022 May 19;10:912178.
34. Bhatti GK, Bhatti JS, Kiran R, Sandhir R. Alterations in Ca2+ homeostasis and oxidative damage induced by ethion in erythrocytes of Wistar rats: ameliorative effect of vitamin E. Environmental toxicology and pharmacology. 2011 May 1;31(3):378-86.
35. Priya RS, Geetha D, Ramesh PS. Antioxidant activity of chemically synthesized AgNPs and biosynthesized *Pongamia pinnata* leaf extract mediated AgNPs–A comparative study. Ecotoxicology and environmental safety. 2016 Dec 1;134:308-18.
36. Reshi MS, Uthra C, Yadav D, Sharma S, Singh A, Sharma A. Silver nanoparticles protect acetaminophen induced acute hepatotoxicity: a biochemical and histopathological approach. Regulatory Toxicology and Pharmacology. 2017 Nov 1;90:36-41.
37. Elfaky MA, Sirwi A, Ismail SH, Awad HH, Gad SS. Hepatoprotective effect of silver nanoparticles at two different particle sizes: Comparative study with and without silymarin. Current Issues in Molecular Biology. 2022 Jun 30;44(7):2923-38.
38. Rana SV. A Comprehensive Assessment of Hepatotoxicity Induced by Engineered Nanoparticles-A Review. J. Toxicol. Risk Assess. 2020;6:035.
39. Kaushal A, Khurana I, Yadav P, Allawadhi P, Banothu AK, Neeradi D, Thalugula S. Advances in therapeutic applications of silver nanoparticles. Chemico-Biological Interactions. 2023 Sep 1;382:110590.
40. Ranjith D, Padhi PK, Madhu CL, Telang AG, Kumar D. Acute Oral Toxicity Study of Ethion in Male Wistar Albino Rats. Journal of Veterinary Pharmacology and Toxicology. 2022;21(2):59-65.
41. Shukla A, Telang AG, Tiwari S, Kumar T, Choudhury S, Kumar D. Elucidation of rhythmic alterations in hepatological status between two fortnight intervals through oxidative damage by ethion in indigenous chicks. Biological Rhythm Research. 2021 Jul 3;52(6):895-909.
42. Bhatti GK, Sidhu IP, Saini NK, Puar SK, Singh G, Bhatti JS. Ameliorative role of melatonin against cypermethrin induced hepatotoxicity and impaired antioxidant defense system in Wistar rats. IOSR J Environ Sci Toxicol Food Technol. 2014 Feb;8(1):39-48.
43. Kaushik U, Joshi SC. Assessment of size based oral toxicity of silver nanoparticles on serum lipid profile, liver and kidney function of male Wistar rats. World J Pharm Res. 2016 Aug 5;5(10):786-803.
44. Kim S, Choi JE, Choi J, Chung KH, Park K, Yi J. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicology in vitro. 2009 Sep 1;23(6):1076-84.
45. Piao MJ, Kang KA, Lee IK, Kim HS, Kim S, Choi JY. Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis. Toxicology letters. 2011 Feb 25;201(1):92-100.
46. Shukla A, Telang AG, Tiwari S, Kumar T, Choudhury S, Kumar D. Exposure to ethion alters rhythmic dynamicity of milieu interior of poultry following exposure at cage system of rearing in Ruhelkhand climatic zone. Biological Rhythm Research. 2022 May 4;53(5):676-88.
47. Sulaiman FA, Adeyemi OS, Akanji MA, Oloyede HO, Sulaiman AA, Olatunde A. Biochemical and morphological alterations caused by silver nanoparticles in Wistar rats. Journal of Acute Medicine. 2015 Dec 1;5(4):96-102.
48. Elle RE, Gaillet S, Vidé J, Romain C, Lauret C, Rugani N. Dietary exposure to silver nanoparticles in Sprague–Dawley rats: effects on oxidative stress and inflammation. Food and chemical toxicology. 2013 Oct 1;60:297-301.
49. El Mahdy MM, Eldin TA, Aly HS, Mohammed FF, Shaalan MI. Evaluation of hepatotoxic and genotoxic potential of silver nanoparticles in albino rats. Experimental and toxicologic pathology. 2015 Jan 1;67(1):21-9.