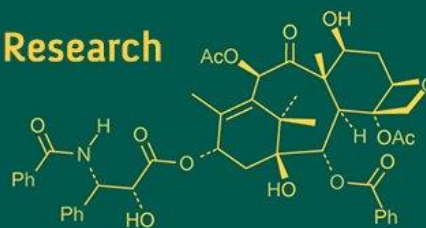


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2026): 5.29

IJABR 2026; 10(7): 1097-1103

www.biochemjournal.com

Received: 14-05-2026

Accepted: 18-06-2026

Surjith KP

Assistant Professor, Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Pookode, KVASU, Wayanad, Kerala, India

Ranjith D

Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, KVASU, Pookode, Wayanad, Kerala, India

Rajani CV

Professor, Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, KVASU, Pookode, Wayanad, Kerala, India

Patki HS

Assistant Professor, Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Pookode, KVASU, Wayanad, Kerala, India

Maya S

Professor, Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Mannuthy, KVASU, Pookode, Wayanad, Kerala, India

Indu VR

Professor, Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Mannuthy, KVASU, Pookode, Wayanad, Kerala, India

Aswathi PB

Assistant Professor, Department of Poultry Science, College of Veterinary and Animal Sciences, KVASU, Pookode, Wayanad, Kerala, India

Pradeep M

Associate Professor, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, KVASU, Pookode, Wayanad, Kerala, India

Corresponding Author:

Surjith KP

Assistant Professor, Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Pookode, KVASU, Wayanad, Kerala, India

Impact of ethion and silver nanoparticles on oxidative stress markers in developing avian embryos: A dose-dependent analysis

Surjith KP, Ranjith D, Rajani CV, Patki HS, Maya S, Indu VR, Aswathi PB and Pradeep M

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7n.9331>

Abstract

The study investigated the effects of ethion, a widely used organophosphate pesticide, and silver nanoparticles (AgNPs) on oxidative stress markers in developing avian embryos. Ethion exposure significantly increased lipid peroxidation, indicated by elevated malondialdehyde levels, and concurrently decreased the activities of key antioxidant enzymes catalase, superoxide dismutase, glutathione (GSH), and glutathione peroxidase (GPx). Treatment of ethion-exposed groups with low to moderate doses of silver nanoparticles demonstrated an ameliorative effect on oxidative stress induced by ethion, normalizing the levels of these enzymes. However, high doses of silver nanoparticles exacerbated lipid peroxidation and reduced enzyme activities, highlighting a complex dose-dependent interaction. These findings underscore the potential of silver nanoparticles as protective agents against pesticide toxicity, yet caution against their excessive use. Further investigations are needed to elucidate the mechanisms underlying these interactions and to optimize dosing strategies for safer agricultural applications.

Keywords: Ethion, silver nanoparticles (AgNPs), oxidative stress

1. Introduction

Pesticides play a critical role in modern agriculture, aiding in the control of pests, weeds, and diseases, thereby enhancing crop yields and ensuring food security (Anani *et al.*, 2020) [1]. Over the past decades, the global use of pesticides has surged, with recent statistics indicating that more than 2 million tons are applied worldwide annually (Sharma *et al.*, 2019) [2]. Among the different classes, organophosphates (OPs) remain one of the most frequently used, accounting for about 40% of all pesticides applied, mainly due to their effectiveness, relatively low persistence in the environment, and reduced risk of bioaccumulation compared to older pesticide classes like organochlorines (Goeb *et al.*, 2020) [3].

Ethion, a widely used organophosphate insecticide, is primarily employed to protect crops such as citrus fruits, cotton, and vegetables from a variety of pests (Ahmad, 2008) [4]. Although effective, ethion poses significant risks to non-target organisms, including birds, fish, and mammals (Roney *et al.*, 2000) [5]. The toxicological effects of ethion are primarily attributed to its ability to inhibit acetylcholinesterase activity, leading to the accumulation of acetylcholine at nerve synapses (Dewan *et al.*, 2008) [6]. This, in turn, affects the nervous system and can lead to various health issues. Importantly, ethion has also been shown to induce oxidative stress by generating reactive oxygen species (ROS) that cause damage to cellular components and disrupt the balance of antioxidant defense mechanisms, leading to alterations in crucial antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) (Singh *et al.*, 2006) [7]. Understanding these effects is vital for assessing the potential impact of ethion on the health of exposed organisms (Foster *et al.*, 2004) [8].

Silver nanoparticles (AgNPs) have emerged as a versatile nanomaterial with a wide range of applications due to their excellent antimicrobial, antifungal, and antiviral properties (Galdiero *et al.*, 2011) [9]. In agriculture, AgNPs are increasingly used to combat plant pathogens, reduce crop diseases, and improve growth, acting as nano-fertilizers or protective agents (Anand and Bhagat, 2019) [10].

Their use in animal husbandry has also been explored, where they help improve livestock health and prevent infections, often serving as a potential alternative to conventional antibiotics (Franci *et al.*, 2015) ^[11]. Additionally, they have shown promise in enhancing feed efficiency and supporting overall animal productivity (Saleh and El-Magd, 2018) ^[12].

Despite these advantages, excessive exposure to silver nanoparticles can pose health risks. At high concentrations, AgNPs can disrupt cellular functions, induce oxidative stress, and impair antioxidant defense systems, leading to cellular toxicity (Kim and Ryu, 2013) ^[13]. This toxicity is of particular concern because it may damage vital organs, such as the liver and kidneys, by generating reactive oxygen species (ROS) and affecting overall redox balance (Wang *et al.*, 2017) ^[14]. Hence, understanding the dose-dependent effects of silver nanoparticles is crucial when considering their application, especially in combination with pesticides like ethion (Tiwari *et al.*, 2011) ^[15].

Therefore, the present study was carried out to investigate the effects of ethion and silver nanoparticles on oxidative stress markers in developing avian embryos (Patlolla *et al.*, 2015; Bhatti *et al.*, 2011) ^[16, 17]. The aim was to evaluate how exposure to ethion, either alone or in combination with silver nanoparticles, impacts key oxidative stress biomarkers such as lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx) (Wen *et al.*, 2022) ^[18]. Understanding these effects is essential, as oxidative stress plays a significant role in cellular damage, leading to potential toxicity and developmental abnormalities in avian embryos (Rezaei *et al.*, 2018) ^[19]. By assessing the changes in these oxidative stress markers, this study sought to elucidate the extent to which ethion induces oxidative damage and how silver nanoparticles, at varying doses, either ameliorate or exacerbate this damage (Reshi *et al.*, 2017; Elfaky *et al.*, 2022) ^[20, 21]. The findings from this research will provide valuable insights into the potential protective or toxic effects of silver nanoparticles against pesticide-induced oxidative stress, contributing to a better understanding of their combined impact on avian development (Nasir *et al.*, 2015) ^[22].

2. Materials and Methods

2.1 Chemicals and Reagents

Ethion was obtained from Cheminova Industries Pvt. Ltd. (Mumbai, India), while all other chemicals were of analytical grade and sourced from reputable suppliers. Silver nanoparticles (AgNPs) were synthesized following the method described by (Yerragopu *et al.*, 2020) ^[23].

2.2 Egg Procurement and Preliminary Preparation

Fertile White Leghorn eggs were acquired from the Instructional Livestock Farm Complex (ILFC) at the College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, following ethical approval (IAEC/COVAS/PKD/21/01/2023 dated 12.07.2023). The eggs were cleaned with 70% alcohol, allowed to air dry, weighed to approximately 50±5 grams, and appropriately labelled. Defective eggs were identified and removed by candling, and the air cell position was marked. The eggs were then incubated at 37.8°C with 55% humidity and turned every four hours (Adame and Ameha, 2023) ^[24].

2.3 Yolk Sac Injection Method

On the fifth day of incubation, 120 eggs were candled to clearly identify the vitelline vessels. Each egg was injected with a 100 µL solution containing ethion, silver nanoparticles, or their combinations directly into the yolk sac (Bhanja *et al.*, 2004) ^[25]. The eggs were distributed into ten distinct groups: a control group, groups treated with silver nanoparticles, groups treated with ethion, and groups receiving their combinations. Details of the experimental grouping are provided in the table below.

2.4 Experimental Design and Group Allocation

The study involved 10 groups of 12 embryonated eggs, to investigate the effects of silver nanoparticles and ethion, individually and in combination, on avian embryo development. Group 1 served as the control and received 100 µL of olive oil. Groups 2, 3, and 4 were treated with silver nanoparticles at doses of 25, 50, and 100 µg/egg, respectively, while Groups 5, 6, and 7 received ethion at doses of 73.5, 147, and 294 µg/egg in 100 µL olive oil. The acute toxicity of ethion in avian embryos was assessed on the fifth day of incubation using the method outlined by Randhawa (2009) ^[26]. Fifty embryos were divided into five groups, each containing ten embryos, and five different doses of ethion, ranging from 0.5 to 3.5 mg/egg, were administered via the yolk sac route. Each dose was delivered in a constant volume of 100 µL per egg. The embryos were observed for mortality at 30 minutes, 2 hours, 6 hours, 24 hours, and up to 14 days post-inoculation. Mortality was recorded after every observation, and the percentage of mortality was calculated to determine the LD50 values. Groups 8, 9, and 10 were administered combined treatments: 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. Injections were given on the 5th day of incubation, and the eggs remained incubated until day 19, when they were opened for analysis of oxidative stress biomarker, as detailed in Table 1.

2.5 Assessment of Oxidative Stress Biomarkers

On the 19th day of incubation, liver samples were collected for analysis of oxidative stress biomarkers. A 10% liver homogenate was prepared in RIPA buffer, centrifuged at 3000 rpm for 15 minutes, and the supernatant was used to analyze oxidative stress markers, including lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione reduced (GSH), and glutathione peroxidase (GPx). (Guo *et al.*, 2022) ^[27].

2.6 Statistical Evaluation

The data were presented as Mean ± SEM (n=12). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, utilizing SPSS Version 24.0 software.

3. Results and Discussion

The measurement of oxidative stress parameters is crucial in experimental studies as it provides valuable insights into the cellular and biochemical effects of various substances, including toxins and therapeutics (Milatovic *et al.*, 2006) ^[28]. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, leading to potential damage to lipids, proteins and DNA (Loft and Poulsen, 1999) ^[29]. By assessing markers

such as lipid peroxidation, superoxide dismutase, catalase and glutathione levels, researchers can evaluate the extent of oxidative damage and the effectiveness of antioxidant responses in biological systems (Enkvetchakul *et al.*, 1993) [30]. This is particularly significant in studies of environmental pollutants like pesticides and nanomaterials, as it helps elucidate their mechanisms of toxicity and the potential for protective strategies (Manke *et al.*, 2013) [31]. Understanding oxidative stress dynamics allows for a more comprehensive assessment of health risks and notifies the development of mitigation approaches in both agricultural and medical contexts (Costantini, 2019) [32].

In addition to its acetylcholinesterase (AChE) inhibitory activity, oxidative stress induction plays a pivotal role in the mechanism of action of ethion (Durom *et al.*, 2024) [33]. Ethion, an organophosphate pesticide, exerts its toxic effects not only by disrupting neurotransmission but also by generating reactive oxygen species (ROS) that overwhelm the cellular antioxidant defense system (Shukla *et al.*, 2021) [34]. This oxidative stress can lead to lipid peroxidation, protein oxidation, and DNA damage, contributing to cellular dysfunction and apoptosis (Bhatti *et al.*, 2011) [17]. Elevation of ROS levels are linked to various pathological conditions, including neurodegenerative diseases and developmental disorders in embryos (Micangeli *et al.*, 2022) [36]. Consequently, the oxidative stress induced by ethion may exacerbate its neurotoxic effects, leading to more severe developmental consequences in avian embryos (Ranjith *et al.*, 2024) [38]. Understanding this dual mechanism enhances our comprehension of ethion's toxicity and underscores the need to evaluate both its enzymatic inhibition and oxidative stress contributions in toxicological assessments (Cortés-Iza and Rodríguez, 2018) [37].

Ethion administration significantly increases lipid peroxidation (Table 2), as indicated by the elevated levels of malondialdehyde (MDA), a key byproduct of lipid oxidation (Bhatti *et al.*, 2010) [39]. This occurs primarily through the generation of reactive oxygen species (ROS) that result from ethion's toxic effects (Tuncmen and Tüzmen, 2007) [40]. When ethion is metabolized, it can lead to an imbalance between the production of ROS and the antioxidant defenses, resulting in oxidative stress (Guendouz *et al.*, 2023) [42]. This oxidative stress initiates the peroxidation of polyunsaturated fatty acids in cell membranes, leading to increased MDA formation (Bhatti *et al.*, 2011) [17].

At low and moderate doses of ethion, the co-administration of silver nanoparticles (AgNPs) appears to have a protective effect, likely due to the nanoparticles' potential antioxidant properties (Reshi *et al.*, 2017) [20]. Silver nanoparticles can scavenge ROS, reducing oxidative stress and thus limiting lipid peroxidation (Reshi *et al.*, 2020) [44]. This suggests that at these lower doses, AgNPs may enhance the embryo's antioxidant defenses, leading to no significant change or even amelioration of ethion's toxic effects (Sreelekha *et al.*, 2021) [45]. However, at high doses of AgNPs, the situation changes. High concentrations of AgNPs may exert pro-oxidant effects, overwhelming the antioxidant capacity and contributing to further oxidative stress (Kim and Ryu, 2013) [13]. This could result from increased synthesis of ROS or from the saturation of the antioxidant systems, leading to increased lipid peroxidation and higher levels of MDA (Khan *et al.*, 2017) [47]. The combination of high-dose AgNPs with high-dose ethion exacerbates the oxidative stress, resulting in an additive or synergistic toxic effect that

which manifests as increased lipid peroxidation (Özkul *et al.*, 2021) [48].

Reduced Glutathione (GSH) in the present study showed a dose-dependent reduction in ethion and AgNP-treated groups when compared to control groups (Table 2). Reduced Glutathione (GSH) is a tripeptide composed of glutamine, cysteine and glycine, and it plays a pivotal role as a major antioxidant in cells (Hussain *et al.*, 1999) [56]. GSH protects cells by neutralizing reactive oxygen species (ROS) and maintaining the redox state of the cell. It acts as a substrate for various enzymes and is crucial for detoxification processes (Yuan and Kaplowitz, 2009) [57].

Superoxide Dismutase (SOD) catalyzes the dismutation of superoxide radicals (O_2^-), converting them into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). By doing so, SOD helps to mitigate the harmful effects of superoxide, which can cause cellular damage if allowed to accumulate (Arora *et al.*, 2008) [51].

Catalase further processes hydrogen peroxide produced by SOD, converting it into water (H_2O) and oxygen (O_2). This enzyme is essential for preventing the accumulation of H_2O_2 , which can also lead to oxidative damage if levels are not adequately controlled (Docea *et al.*, 2020) [52].

In the ethion-treated group, the observed decrease in both catalase and SOD levels indicates that ethion induces oxidative stress, overwhelming the antioxidant defense system (Bhatti *et al.*, 2011). Ethion may promote the production of reactive oxygen species (ROS), which can damage cellular components and hinder the normal expression or activity of these protective enzymes (Singh *et al.*, 2006) [7].

In the groups co-administered with low and moderate doses of silver nanoparticles alongside ethion, the normalization of catalase and SOD levels suggests that the silver nanoparticles enhance the antioxidant capacity of the developing embryos (Reshi *et al.*, 2020) [44]. By reducing oxidative stress, AgNPs help restore the activity of these enzymes, allowing them to function effectively in mitigating further oxidative damage (Sreelekha *et al.*, 2021) [45].

However, in the groups receiving high doses of silver nanoparticles, both catalase and SOD levels decrease again (Shayesteh *et al.*, 2014) [53]. This could indicate that excessive silver nanoparticles may induce pro-oxidative effects, leading to further ROS production that overwhelms the antioxidant defences (Gan *et al.*, 2020) [54]. When combined with high doses of ethion, the resulting oxidative stress may be too severe for the antioxidant systems to cope with resulting in decreased enzyme levels (Abdel-Gawad *et al.*, 2021) [55]. This highlights the importance of dose management in therapeutic applications, as both low and moderate doses can be protective, while high doses can be detrimental (Reshi *et al.*, 2017) [20].

Glutathione Peroxidase (GPx) is an enzyme that catalyzes the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides, using GSH as a reducing agent (Ben Amara *et al.*, 2015) [58]. This reaction converts H_2O_2 into water, thereby protecting the cell from oxidative damage (Little *et al.*, 1970) [59].

In the ethion-treated group, the decrease in both GSH and GPx levels indicates that ethion exposure induces oxidative stress, leading to glutathione depletion and reduced GPx activity (Bhatti *et al.*, 2010) [39]. This suggests that ethion may either enhance the production of ROS or interfere with

the synthesis and recycling of GSH, compromising the antioxidant defense (Singh *et al.*, 2006) [7].

In groups receiving low and moderate doses of (AgNPs) alongside ethion, the restoration of GSH and GPx levels implies that AgNPs have a protective effect against oxidative stress (Reshi *et al.*, 2020) [44]. They may enhance the synthesis or regeneration of GSH, thereby allowing GPx to function effectively and reduce hydrogen peroxide levels (Reshi *et al.*, 2017) [20].

However, in the high-dose silver nanoparticles groups, both GSH and GPx levels decline again (Ranjbar *et al.*, 2014) [60]. This could suggest that at elevated doses, AgNPs may exert a pro-oxidative effect, leading to increased ROS that depletes GSH and overwhelms GPx (Yin *et al.*, 2013) [61]. When combined with high doses of ethion, cumulative oxidative stress may exceed the antioxidant system's capacity, resulting in decreased levels of these critical enzymes (Shukla *et al.*, 2021) [34]. This highlights the need for careful dosage considerations, as both insufficient and excessive doses can impair the antioxidant response (Ranjith *et al.*, 2022) [62].

Overall, the differing effects observed in low versus high

doses of silver nanoparticles highlight the complex interplay between dose-dependent responses and the mechanisms of oxidative stress (Park *et al.*, 2010) [49]. While lower doses may provide protective benefits, higher doses can exacerbate oxidative damage, underscoring the importance of dose considerations in toxicological studies (Williams *et al.*, 2016) [50].

In summary, the study investigated the effects of ethion and silver nanoparticles (AgNPs) on oxidative stress markers in developing avian embryos. Ethion exposure significantly elevated lipid peroxidation, indicated by increased malondialdehyde levels, reflecting enhanced oxidative stress. While low to moderate doses of AgNPs co-administered with ethion normalized oxidative stress markers and mitigated ethion's toxic effects, high doses of AgNPs exacerbated lipid peroxidation and decreased antioxidant enzyme levels, including catalase, superoxide dismutase, glutathione, and glutathione peroxidase. These findings underscore the dual role of AgNPs as both protective agents at lower doses and potential stressors at higher concentrations, emphasizing the importance of dosage in managing oxidative stress in avian embryos.

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 (<i>In-ovo</i>)	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@ E _{1/20})	73.5 µg/egg in 100 µL of olive oil	12
6	Ethion (ETH-147@ E _{1/10})	147 µg/egg in 100 µL of olive oil	12
7	Ethion (ETH-294@ E _{1/5})	294 µg/egg in 100 µL of olive oil	12
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

Table 2: Effect of *in ovo* administration of ethion and silver nanoparticles (AgNP) on non-enzymatic oxidative stress markers on 20th day of pre-hatch in White Leghorn embryos

Treatment	LPO (nmol/g protein)	GSH (nmol/g protein)
Control	2.31±0.12 ^{ab}	82.31±1.42 ^e
E _{1/5}	4.76±0.17 ^d	61.53±1.38 ^a
E _{1/10}	4.27±0.16 ^c	67.57±1.62 ^b
E _{1/20}	2.36±0.19 ^b	82.39±1.39 ^e
Ag ₁₀₀	4.38±0.21 ^c	70.57±1.42 ^c
Ag ₅₀	2.30±0.14 ^a	82.49±1.62 ^e
Ag ₂₅	2.30±0.13 ^a	82.33±1.38 ^e
E _{1/5} + Ag ₁₀₀	4.78±0.12 ^d	61.41±1.52 ^a
E _{1/10} + Ag ₅₀	2.32±0.13 ^b	79.92±1.56 ^d
E _{1/20} + Ag ₂₅	2.32±0.12 ^{ab}	82.18±1.58 ^e
F value	5.193*	10.418**
P value	0.021	0.009

LPO- Lipid Peroxidation, GSH-Reduced Glutathione, nmol-nanomoles, mg-milligrams. Values with superscripts indicates statistical significance as determined by one-way ANOVA followed by Tukey's multiple comparison test indicating significance levels of $p < 0.05$.

Table 3: Effect of *in ovo* administration of ethion and silver nanoparticles (AgNP) on enzymatic oxidative stress markers on 20th day of pre-hatch in White Leghorn embryos

Treatment	Catalase (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)
Control	25.87±0.784 ^d	10.14±0.21 ^f	1.52±0.051 ^d
E _{1/5}	11.47±0.785 ^a	6.17±0.25 ^b	0.63±0.06 ^a
E _{1/10}	15.59±0.782 ^b	7.14±0.26 ^c	0.78±0.054 ^b
E _{1/20}	25.74±0.785 ^d	10.19±0.28 ^f	1.53±0.057 ^d
Ag ₁₀₀	17.69±0.795 ^c	7.29±0.31 ^d	0.85±0.052 ^c
Ag ₅₀	25.77±0.792 ^d	10.02±0.28 ^e	1.52±0.056 ^d
Ag ₂₅	25.93±0.795 ^d	10.01±0.29 ^e	1.52±0.052 ^d
E _{1/5} + Ag ₁₀₀	11.28±0.725 ^a	6.07±0.31 ^a	0.63±0.054 ^a
E _{1/10} + Ag ₅₀	25.89±0.726 ^d	10.05±0.30 ^e	1.52±0.053 ^d
E _{1/20} + Ag ₂₅	25.81±0.825 ^d	10.21±0.31 ^f	1.52±0.051 ^d
F value	5.428*	5.237*	10.185**
P value	0.021	0.018	0.008

SOD- Superoxide Dismutase, GPx-Glutathione Reductase, U-Units, mg-Milligrams. Values with superscripts indicates statistical significance as determined by one-way ANOVA followed by Tukey's multiple comparison test indicating significance levels of $p < 0.05$.

4. Conclusion

In conclusion, this study highlights the detrimental impact of ethion on oxidative stress markers in developing avian embryos, demonstrating increased lipid peroxidation and reduced activity of key antioxidant enzymes. The use of silver nanoparticles revealed a complex, dose-dependent interaction: low to moderate doses were effective in ameliorating the toxic effects of ethion, whereas higher doses led to exacerbated oxidative stress. These findings suggest that while silver nanoparticles hold promise as protective agents against pesticide toxicity, their application must be approached with caution to avoid adverse effects. Further research is warranted to explore optimal dosing strategies and the mechanisms underlying these interactions, thereby contributing to safer agricultural practices and enhanced animal health.

References

- Anani OA, Mishra RR, Mishra P, Enuneku AA, Anani GA, Adetunji CO. Effects of toxicant from pesticides on food security: current developments. In: Kothari SL, Kumar S, Sharma Shiv M, editors. Innovations in food technology: current perspectives and future goals. Singapore: Springer Singapore; 2020. p. 313-321. https://doi.org/10.1007/978-981-15-4127-8_25
- Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GP, Handa N, *et al.* Worldwide pesticide usage and its impacts on ecosystem. SN Appl Sci. 2019;1(11):1446. <https://doi.org/10.1007/s42452-019-1485-1>
- Goeb J, Dillon A, Lupi F, Tschirley D. Pesticides: what you don't know can hurt you. J Assoc Environ Resour Econ. 2020;7(5):801-836. <https://doi.org/10.1086/709141>
- Ahmad M. Potentiation between pyrethroid and organophosphate insecticides in resistant field populations of cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. Pestic Biochem Physiol. 2008;91(1):24-31. <https://doi.org/10.1016/j.pestbp.2007.12.002>
- Roney N, Osier MR, Avallone AD. Toxicological profile for ethion. Atlanta (GA): Agency for Toxic Substances and Disease Registry; 2000.
- Dewan A, Patel AB, Pal RR, Jani UJ, Singel VC, Panchal MD. Mass ethion poisoning with high mortality. Clin Toxicol. 2008;46(1):85-88. <https://doi.org/10.1080/15563650701460334>
- Singh M, Sandhir R, Kiran R. Erythrocyte antioxidant enzymes in toxicological evaluation of commonly used organophosphate pesticides. Indian J Exp Biol. 2006;44(7):580-583.
- Foster LJ, Kwan BH, Vancov T. Microbial degradation of the organophosphate pesticide, ethion. FEMS Microbiol Lett. 2004;240(1):49-53. <https://doi.org/10.1016/j.femsle.2004.09.011>
- Galdiero S, Falanga A, Vitiello M, Cantisani M, Marra V, Galdiero M. Silver nanoparticles as potential antiviral agents. Molecules. 2011;16(10):8894-8918. <https://doi.org/10.3390/molecules16108894>
- Anand R, Bhagat M. Silver nanoparticles (AgNPs): as nanopesticides and nanofertilizers. MOJ Biol Med. 2019;4(1):19-20. <https://doi.org/10.15406/mojbm.2019.04.00110>
- Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, *et al.* Silver nanoparticles as potential antibacterial agents. Molecules. 2015;20(5):8856-8874. <https://doi.org/10.3390/molecules20058856>
- Saleh AA, El-Magd MA. Beneficial effects of dietary silver nanoparticles and silver nitrate on broiler nutrition. Environ Sci Pollut Res. 2018;25(27):27031-27038. <https://doi.org/10.1007/s11356-018-2742-1>
- Kim S, Ryu DY. Silver nanoparticle-induced oxidative stress, genotoxicity and apoptosis in cultured cells and animal tissues. J Appl Toxicol. 2013;33(2):78-89. <https://doi.org/10.1002/jat.2792>
- Wang E, Huang Y, Du Q, Sun Y. Silver nanoparticle induced toxicity to human sperm by increasing ROS (reactive oxygen species) production and DNA damage. Environ Toxicol Pharmacol. 2017;52:193-199. <https://doi.org/10.1016/j.etap.2017.04.010>
- Tiwari DK, Jin T, Behari J. Dose-dependent in-vivo toxicity assessment of silver nanoparticle in Wistar rats. Toxicol Mech Methods. 2011;21(1):13-24. <https://doi.org/10.3109/15376516.2010.529184>
- Patlolla AK, Hackett D, Tchounwou PB. Silver nanoparticle-induced oxidative stress-dependent toxicity in Sprague-Dawley rats. Mol Cell Biochem. 2015;399(1-2):257-268. <https://doi.org/10.1007/s11010-014-2252-7>
- Bhatti GK, Bhatti JS, Kiran R, Sandhir R. Biochemical and morphological perturbations in rat erythrocytes exposed to ethion: protective effect of vitamin E. Cell Mol Biol (Noisy-le-grand). 2011;57(1):70-79.
- 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. Front Bioeng Biotechnol. 2022;10:912178. <https://doi.org/10.3389/fbioe.2022.912178>
- Rezaei A, Farzinpour A, Vaziry A, Jalili A. Effects of silver nanoparticles on hematological parameters and hepatorenal functions in laying Japanese quails. Biol Trace Elem Res. 2018;185(2):475-485. <https://doi.org/10.1007/s12011-018-1262-6>
- Reshi MS, Uthra C, Yadav D, Sharma S, Singh A, Sharma A, *et al.* Silver nanoparticles protect acetaminophen induced acute hepatotoxicity: a biochemical and histopathological approach. Regul Toxicol Pharmacol. 2017;90:36-41. <https://doi.org/10.1016/j.yrtph.2017.08.009>
- 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. Curr Issues Mol Biol. 2022;44(7):2923-2938. <https://doi.org/10.3390/cimb44070202>
- Nasir GA, Samir HF, Mohammed AK. Effects of silver nanoparticles in liver function enzymes and oxidative stress levels. World J Pharm Res. 2015;4(11):2080-2089.
- 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. Int Res J Pure Appl Chem. 2020;21(3):37-50. <https://doi.org/10.9734/irjpac/2020/v21i330164>
- Adame MM, Ameha N. Review on egg handling and management of incubation and hatchery environment.

- Asian J Biol Sci. 2023;16(4):474-484. <https://doi.org/10.3923/ajbs.2023.474.484>
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 J Poult Sci. 2004;39(2):105-111.
 26. Randhawa MA. Calculation of LD50 values from the method of Miller and Tainter, 1944. J Ayub Med Coll Abbottabad. 2009;21(3):184-185.
 27. Guo J, Yan WR, Tang JK, Jin X, Xue HH, Wang T, *et al.* Dietary phillygenin supplementation ameliorates aflatoxin B1-induced oxidative stress, inflammation, and apoptosis in chicken liver. Ecotoxicol Environ Saf. 2022;236:113481. <https://doi.org/10.1016/j.ecoenv.2022.113481>
 28. Milatovic D, Gupta RC, Aschner M. Anticholinesterase toxicity and oxidative stress. ScientificWorldJournal. 2006;6:295-310. <https://doi.org/10.1100/tsw.2006.38>
 29. Loft S, Poulsen HE. Markers of oxidative damage to DNA: antioxidants and molecular damage. Methods Enzymol. 1999;300:166-184. [https://doi.org/10.1016/s0076-6879\(99\)00080-8](https://doi.org/10.1016/s0076-6879(99)00080-8)
 30. Enkvetchakul B, Bottje W, Anthony N, Moore R, Huff W. Compromised antioxidant status associated with ascites in broilers. Poult Sci. 1993;72(12):2272-2280. <https://doi.org/10.3382/ps.072272>
 31. Manke A, Wang L, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. BioMed Res Int. 2013;2013:942916. <https://doi.org/10.1155/2013/942916>
 32. Costantini D. Understanding diversity in oxidative status and oxidative stress: the opportunities and challenges ahead. J Exp Biol. 2019;222(13):jeb194688. <https://doi.org/10.1242/jeb.194688>
 33. Durom EG, Aneesha VA, Kumar NV, Azeez AB, Karikalan M, Lingaraju MC, *et al.* Prenatal exposure to ethion caused maternal and foetal toxicity in rats. Reprod Toxicol. 2024;126:108607. <https://doi.org/10.1016/j.reprotox.2024.108607>
 34. 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. Biol Rhythm Res. 2021;52(6):895-909. <https://doi.org/10.1080/09291016.2019.1628372>
 35. Bhatti GK, Bhatti JS, Kiran R, Sandhir R. Biochemical and morphological perturbations in rat erythrocytes exposed to ethion: protective effect of vitamin E. Cell Mol Biol (Noisy-le-grand). 2011;57(1):70-79.
 36. Micangeli G, Menghi M, Profeta G, Tarani F, Mariani A, Petrella C, *et al.* The impact of oxidative stress on pediatrics syndromes. Antioxidants. 2022;11(10):1983. <https://doi.org/10.3390/antiox11101983>
 37. Cortés-Iza SC, Rodríguez AI. Oxidative stress and pesticide disease: a challenge for toxicology. Rev Fac Med. 2018;66(2):261-267. <https://doi.org/10.15446/revfacmed.v66n2.60623>
 38. Ranjith D, Telang AG, Subhadra S, Kamothi DJ, Madhu CL, Kumar D. Chitosan-TPP encapsulated quercetin nanoparticles: amplified protection mechanisms unveiled against ethion-induced developmental toxicity through comprehensive in-vivo and in-silico elucidation. Toxicol Res (Camb). 2024;13(3):tfac074. <https://doi.org/10.1093/toxres/tfac074>
 39. Bhatti GK, Kiran R, Sandhir R. Modulation of ethion-induced hepatotoxicity and oxidative stress by vitamin E supplementation in male Wistar rats. Pestic Biochem Physiol. 2010;98(1):26-32. <https://doi.org/10.1016/j.pestbp.2010.04.008>
 40. Tuncmen H, Tuzmen MN. Biochemical effects of pesticide contaminated drinking water on lipid peroxidation and free-radical scavenger. Hacettepe J Biol Chem. 2007;35(2):111-116.
 41. 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. Environ Toxicol Pharmacol. 2011;31(3):378-386. <https://doi.org/10.1016/j.etap.2011.01.008>
 42. Guendouz A, Belguet A, Dahamna S. The estimation of lipid peroxidation and osmotic fragility of human red blood cell induced by pesticides residues detected in some vegetables crops. Asian J Dairy Food Res. 2023;42(3):383-388. <https://doi.org/10.18805/ajdfr.DR-2015>
 43. Reshi MS, Uthra C, Yadav D, Sharma S, Singh A, Sharma A, *et al.* Silver nanoparticles protect acetaminophen induced acute hepatotoxicity: a biochemical and histopathological approach. Regul Toxicol Pharmacol. 2017;90:36-41. <https://doi.org/10.1016/j.yrtph.2017.08.009>
 44. Reshi MS, Yadav D, Uthra C, Shrivastava S, Shukla S. Acetaminophen-induced renal toxicity: preventive effect of silver nanoparticles. Toxicol Res (Camb). 2020;9(4):406-412. <https://doi.org/10.1093/toxres/tfaa038>
 45. Sreelekha E, George B, Shyam A, Sajina N, Mathew B. A comparative study on the synthesis, characterization, and antioxidant activity of green and chemically synthesized silver nanoparticles. J Bionanosci. 2021;11(2):489-496.
 46. Kim S, Ryu DY. Silver nanoparticle-induced oxidative stress, genotoxicity and apoptosis in cultured cells and animal tissues. J Appl Toxicol. 2013;33(2):78-89. <https://doi.org/10.1002/jat.2792>
 47. Khan MS, Qureshi NA, Jabeen F, Asghar MS, Shakeel M, Fakhar-e-Alam M. Eco-friendly synthesis of silver nanoparticles through economical methods and assessment of toxicity through oxidative stress analysis in the *Labeo rohita*. Biol Trace Elem Res. 2017;176(2):416-428. <https://doi.org/10.1007/s12011-016-0832-6>
 48. Ozkul T, Gunal SY, Aydogan S. The effect of ethion on erythrocyte deformability. Pestic Biochem Physiol. 2021;172:104753. <https://doi.org/10.1016/j.pestbp.2020.104753>
 49. Park EJ, Bae E, Yi J, Kim Y, Choi K, Lee SH, *et al.* Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. Environ Toxicol Pharmacol. 2010;30(2):162-168. <https://doi.org/10.1016/j.etap.2010.05.004>
 50. Williams KM, Gokulan K, Cerniglia CE, Khare S. Size and dose dependent effects of silver nanoparticle exposure on intestinal permeability in an *in vitro* model of the human gut epithelium. J Nanobiotechnology.

- 2016;14(1):62. <https://doi.org/10.1186/s12951-016-0214-4>
51. Arora S, Jain J, Rajwade JM, Paknikar KM. Cellular responses induced by silver nanoparticles: *in vitro* studies. *Toxicol Lett.* 2008;179(2):93-100. <https://doi.org/10.1016/j.toxlet.2008.04.009>
52. Docea AO, Calina D, Buga AM, Zlatian O, Paoliello MM, Mogosanu GD, *et al.* The effect of silver nanoparticles on antioxidant/pro-oxidant balance in a murine model. *Int J Mol Sci.* 2020;21(4):1233. <https://doi.org/10.3390/ijms21041233>
53. Shayesteh TH, Zijoud H, Ranjbar A. Effects of silver nanoparticle (Ag NP) on oxidative stress, liver function in rat: hepatotoxic or hepatoprotective. *J Issues.* 2014;2(9):158-163.
54. Gan J, Sun J, Chang X, Li W, Li J, Niu S, *et al.* Biodistribution and organ oxidative damage following 28 days oral administration of nanosilver with/without coating in mice. *J Appl Toxicol.* 2020;40(6):815-831. <https://doi.org/10.1002/jat.3946>
55. Abdel-Gawad H, Soliman S, Taha H, Aly M, Abd El Kader MA, Hegazi B. Pathogenic effects of ethion residues and the expected protective role of the ethanolic extract of rosemary (*Rosmarinus officinalis* L.) leaves in male rats. *Egypt J Chem.* 2021;64(4):1817-1829. <https://doi.org/10.21608/ejchem.2021.54714.3138>
56. Hussain S, Atkinson A, Thompson SJ, Khan AT. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver, and kidneys of mice. *J Environ Sci Health B.* 1999;34(4):645-660. <https://doi.org/10.1080/03601239909373218>
57. Yuan L, Kaplowitz N. Glutathione in liver diseases and hepatotoxicity. *Mol Aspects Med.* 2009;30(1-2):29-41. <https://doi.org/10.1016/j.mam.2008.08.003>
58. Ben Amara I, Ben Saad H, Hamdaoui L, Karray A, Boudawara T, Ben Ali Y, *et al.* Maneb disturbs expression of superoxide dismutase and glutathione peroxidase, increases reactive oxygen species production, and induces genotoxicity in liver of adult mice. *Environ Sci Pollut Res.* 2015;22(16):12309-12322. <https://doi.org/10.1007/s11356-015-4518-9>
59. Little C, Olinescu R, Reid KG, O'Brien PJ. Properties and regulation of glutathione peroxidase. *J Biol Chem.* 1970;245(14):3632-3636.
60. Ranjbar A, Ataie Z, Khajavi F, Ghasemi H. Effects of silver nanoparticle (Ag NP) on oxidative stress biomarkers in rat. *J Nanomed.* 2014;1(3):205-210.
61. Yin N, Liu Q, Liu J, He B, Cui L, Li Z, *et al.* Silver nanoparticle exposure attenuates the viability of rat cerebellum granule cells through apoptosis coupled to oxidative stress. *Small.* 2013;9(9-10):1831-1841. <https://doi.org/10.1002/sml.201202271>
62. Ranjith D, Padhi PK, Madhu CL, Telang AG, Kumar D. Acute oral toxicity study of ethion in male Wistar albino rats. *J Vet Pharmacol Toxicol.* 2022;21(2):59-65.