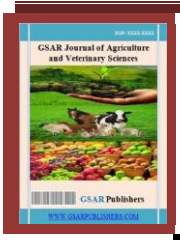

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AMELIORATIVE EFFECT OF FOLIC ACID SUPPLEMENTATION AGAINST IVERMECTIN-INDUCED OXIDATIVE STRESS IN FEMALE RABBITS

By

Baba-Onoja O.M[1], Ndubuisi. D.[2]
[1]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Federal University Wukari.
[2]Department of Animal Science, Faculty of Agriculture, Federal University Wukari

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Abstract

Ivermectin (IVM) is a widely used antiparasitic agent in veterinary medicine; however, repeated administration has been associated with oxidative stress, characterized by elevated malondialdehyde (MDA) levels and depletion of endogenous antioxidants such as reduced glutathione (GSH) and superoxide dismutase (SOD). Folic acid (FA), a B-vitamin with established antioxidant properties, has been shown to reduce oxidative stress through homocysteine-lowering effects, inhibition of NADPH oxidase, and activation of Nrf2-mediated antioxidant defence pathways. This study investigated the ameliorative effect of folic acid supplementation against ivermectin-induced oxidative stress in female rabbits. Nine adult female rabbits were randomly assigned to three groups with 3 animals per group. Group 1served as the control group and they were not treated. Group 2 were administered IVM only at a dosage of 0.1mg/kg subcutaneously weekly for 3 weeks. Group 3 were administered IVM+FA (FA at 10mg/kg) for 21 days. Blood samples were collected and oxidative stress biomarkers including serum MDA, GSH, SOD, catalase (CAT) were assessed at study termination. Results demonstrated that IVM administration significantly increased MDA levels and decreased GSH, SOD, CAT, and TAC compared to controls ($p<0.05$). Concurrent folic acid supplementation significantly attenuated these alterations, restoring oxidative stress markers toward normal levels. These findings indicate that folic acid exerts a protective effect against ivermectin-induced oxidative damage in female rabbits, likely through enhancement of endogenous antioxidant defence mechanisms. This study supports the potential adjunctive use of folic acid to mitigate the oxidative side effects of ivermectin therapy in veterinary practice, particularly in breeding females where reproductive integrity may be compromised by oxidative stress.

Keywords: *Ivermectin, Folic acid, Oxidative stress, Female rabbits, Antioxidant defence*

INTRODUCTION

Ivermectin (IVM), a semisynthetic macrocyclic lactone derived from *Streptomyces avermitilis*, stands as one of the most significant antiparasitic agents in the history of veterinary and human medicine. Since its introduction in the early 1980s, ivermectin has demonstrated remarkable efficacy against a broad spectrum of endoparasites and ectoparasites, including gastrointestinal nematodes, lungworms, mites, and lice. Its mechanism of action involves binding to glutamate-gated chloride channels and gamma-aminobutyric acid (GABA) receptors in the parasite nervous system, leading to increased chloride ion influx, hyperpolarization, and subsequent paralysis and death of the parasite. The drug's high lipid solubility, extensive tissue distribution, and prolonged half-life contribute to its sustained therapeutic efficacy. In rabbit production systems particularly in Nigeria where rabbit farming is growing rapidly, ivermectin is extensively employed for the control of sarcoptic mange caused by *Sarcoptes scabiei* and psoroptic mange due to *Psoroptes cuniculi*, conditions that significantly impair animal welfare, growth performance, and reproductive outcomes (El-shabksky *et al*., 2022).

Despite its undeniable therapeutic benefits, accumulating evidence has raised concerns regarding the oxidative stress-inducing potential of ivermectin, particularly following repeated administration. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the capacity of the endogenous antioxidant defence

*Corresponding Author: **Baba-Onoja O.M.**

system to neutralize these harmful intermediates. Studies in rabbits have demonstrated that repeated administration of ivermectin at therapeutic doses causes significant disturbances in the antioxidant system. Specifically, ivermectin treatment has been associated with increased levels of malondialdehyde (MDA), a reliable biomarker of lipid peroxidation alongside decreased levels of reduced glutathione (GSH) and diminished activity of superoxide dismutase (SOD). Saud *et al.,* (2026) further reported that repeated administration of ivermectin at therapeutic doses at short intervals causes disturbance in blood electrolyte balance, biochemical profile, and antioxidant system in rabbits, and may also negatively impact liver and kidney functions. The mechanistic basis of ivermectin-induced oxidative stress appears multifactorial, involving mitochondrial dysfunction, dysregulated calcium homeostasis, and activation of endoplasmic reticulum stress pathways. In female rabbits, long-term ivermectin administration has been shown to detrimentally affect growth and reproductive performance, effects that are closely linked to oxidative damage.

The vulnerability of female reproductive tissues to oxidative stress is particularly concerning. Ovarian follicles, oocytes, and the uterine environment are highly susceptible to ROS-induced damage, which can compromise oocyte quality, fertilization, embryonic development, and overall fertility. Studies in various species have established that elevated oxidative stress is associated with impaired reproductive outcomes, including reduced conception rates, increased embryonic loss, and diminished litter sizes. In the context of ivermectin therapy, the potential for oxidative damage to compromise reproductive performance in breeding does represents a significant clinical concern that warrants investigation. El-Shobokshy *et al.,* (2022) demonstrated that ivermectin treatment in inorganic selenium-supplemented groups was detrimental to growth and reproductive performance in female rabbits, while these parameters improved in IVM-treated and nano-selenium-supplemented groups, underscoring the potential for antioxidant intervention to mitigate these adverse effects.

Folic acid (FA), also known as vitamin B9 or folate, is an essential water-soluble vitamin that serves as a critical cofactor in one-carbon metabolism, participating in DNA synthesis, repair, and methylation processes. Beyond its well-established role in preventing neural tube defects and supporting cellular proliferation, folic acid has garnered increasing recognition for its antioxidant properties. The antioxidant mechanisms of folic acid are multifaceted and operate through several distinct pathways. Folic acid reduces plasma homocysteine levels, and elevated homocysteine is known to promote oxidative stress through the generation of ROS and impairment of endothelial function. Furthermore, folic acid has been shown to inhibit nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression, thereby reducing superoxide anion production a primary source of vascular oxidative stress. Emerging evidence indicates that folic acid activates the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway, a master regulator of antioxidant defence. Nrf2 activation promotes the expression of multiple antioxidant response element (ARE)-dependent genes, including those encoding glutathione synthesis enzymes, heme oxygenase-1, and NAD(P)H quinone oxidoreductase 1. Studies have demonstrated that folic acid (3 µg/kg) can activate Nrf2 in rats with acute kidney injury, while Nrf2 gene knockout abolished the protective effects of folic acid against oxidative stress in melanocytes. Additionally, folic acid has been demonstrated to enhance the levels of endogenous antioxidants including GSH and total antioxidant capacity while reducing MDA concentrations (Saud *et al.,* 2026).

The protective effects of folic acid against oxidative stress have been documented in various experimental model. In dairy cows subjected to heat stress, dietary folic acid supplementation improved serum and follicular fluid antioxidant capacity (including CAT, GSH-Px, and SOD) while reducing oxidative damage markers such as MDA. Furthermore, studies have demonstrated the protective role of folic acid against ivermectin-induced renal toxicity, where folic acid was shown to protect against ivermectin nephrotoxicity by modulating biomarkers of kidney toxicity, apoptosis, and lipid peroxidation (Saud *et al.,* 2026).

Despite the established roles of ivermectin in inducing oxidative stress and folic acid in providing antioxidant protection, the specific interaction between these two agents in female rabbits has not been systematically investigated. While studies have explored the ameliorative effects of various antioxidants including turmeric extract, vitamins A, D3, E, and H, and nano-selenium against ivermectin-induced oxidative stress in rabbits, the potential of folic acid supplementation to mitigate ivermectin-induced oxidative damage in female rabbits remains underexplored. This knowledge gap is particularly significant given the widespread use of ivermectin in rabbit breeding operations and the critical importance of maintaining reproductive health in does. The female reproductive system, with its high metabolic activity and susceptibility to oxidative injury, represents a particularly relevant target for antioxidant intervention.

The present study was therefore designed to investigate the ameliorative effect of folic acid supplementation against ivermectin-induced oxidative stress in female rabbits. Effects of folic acid on serum levels of MDA (a marker of lipid peroxidation), GSH, SOD, CAT, and total antioxidant capacity in female rabbits receiving repeated ivermectin injections was assessed. This research aims to provide an evidence base for the adjunctive use of this safe, inexpensive, and readily available vitamin to mitigate the oxidative side effects of ivermectin therapy in veterinary practice, ultimately contributing to improved health and reproductive outcomes in breeding female rabbits.

MATERIALS AND METHODS

Experimental Location

The study was carried out at the Federal University Wukari Livestock Teaching and Research Farm located in Wukari Taraba State, Nigeria. Wukari a medium size town with

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coordinates of Latitude 07^0.85' N and longitude 09^0.78' E of the equator.

Experimental Animals

A total of nine (9) apparently healthy grower (3-4 months old) female rabbits were used for this study. The rabbits were sourced from a farmer in Wukari town. Prior to the commencement of the experiment, the rabbits were clinically examined and allowed an acclimatization period of 7 days, during which they were kept under standard management conditions. The experimental trial lasted for of four weeks, during which all treatments were administered and observations were made.

Experimental Design

The experiment was laid out in a completely randomized design (CRD). The nine (9) female rabbits were randomly assigned to three (3) treatment groups, with three (3) rabbits per treatment, as follows:

Group 1 (Control): Rabbits received no treatment

Group 2 (Ivermectin): Rabbits treated with Ivermectin only at 0.1mg/kg body weight

Group 3 (Ivermectin + Folic Acid): Rabbits treated with 0.1mg/kg Ivermectin and supplemented with 10mg/kg of Folic acid.

Management of Experimental Animals

The rabbits were housed individually in clean and well-ventilated cages at the teaching and research farm. They were fed commercial pelleted growers mash and offered clean drinking water *ad libitum* throughout the experimental period. Proper sanitation and routine management practices were maintained to reduce stress and ensure animal welfare.

Drug and Supplement Administration

Ivermectin was administered to rabbits in Group 2 and Group 3 once a week (for three weeks) at a dose of 0.1 mg/kg body weight via the subcutaneous route. Folic acid was administered orally to rabbits in Group 3 only at a dose of 10 mg/kg body weight for the period of 21 days. Rabbits in the control Group 1 did not receive any drug nor supplement.

Sample Collection

At the end of the experimental period, about 5mls of blood samples were collected from each rabbit through the marginal ear vein using sterile syringes. The blood samples were transferred into plain sample bottles and allowed to clot. Serum was obtained by centrifugation and stored at –20°C until analysis.

Determination of Antioxidant Parameters

Serum samples were analysed for antioxidant and oxidative stress parameters using standard laboratory procedures as described by Usman *et al., 2019.* The parameters measured included: Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Malondialdehyde (MDA) as an index of lipid peroxidation.

Statistical Analysis

Data obtained from the experiment were subjected to statistical analysis using Tukey Kramer. Differences among treatment means were considered statistically significant at $p < 0.05$.

RESULT

Table 1: Effect of Folic acid supplementation on Serum Antioxidant Parameters of female rabbits treated with Ivermectin

Parameters	Control	IVM	IVM + F.A	p-value
SOD (U/ml)	57.52 ± 1.1^a	35.50 ± 2.1^b	89.62 ± 2.6^a	0.001
CAT (µg/ml)	130.6 ± 1.6^a	79.97 ± 3.4^b	100.2 ± 3.83^a	0.0003
TAC (µM)	39.65 ± 1.6^a	29.61 ± 1.3^b	44.55 ± 2.2^a	0.0012
MDA (nmol/ml)	7.21 ± 0.3^b	18.02 ± 1.5^a	9.95 ± 0.7^b	0.0011

Key: IVM= Ivermectin, F.A = Folic Acid, SOD= Superoxide dismutase, CAT= Catalase, MDA= Malondialdehyde

Note: ab = means with different superscript are statistically significant ($P<0.05$)

DISCUSSION

The results revealed that IVM administration alone significantly ($P < 0.05$) decreased superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC), while significantly increasing malondialdehyde (MDA) levels compared with the control. However, the inclusion of folic acid alongside IVM (IVM + F.A) restored these parameters to levels comparable with or superior to the control, indicating that folic acid effectively mitigated the oxidative stress induced by ivermectin.

The significant reduction in SOD and CAT activities in the IVM group suggests that ivermectin administration induced oxidative stress by suppressing the primary enzymatic antioxidant defence system. This finding aligns with the work of Okonkwo *et al.* (2023), who reported that ivermectin administration in rabbits led to a marked decrease in hepatic SOD and glutathione peroxidase (GPx) activities, attributing this to the drug's potential to generate reactive oxygen species (ROS) during its biotransformation. Similarly, Adeyemi and Akinlolu (2023) observed that sub-chronic exposure to ivermectin in female Wistar rats significantly downregulated the gene expression of SOD1 and CAT, leading to a compromised antioxidant status. The restoration of these

*Corresponding Author: **Baba-Onoja O.M.**

enzyme activities in the IVM + F.A group to near-control or elevated levels agrees with the findings of Sharma *et al.* (2023), who documented that folic acid supplementation could reverse drug-induced oxidative damage in mammalian models by upregulating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, a master regulator of antioxidant enzymes. The role of folic acid in one-carbon metabolism and its ability to maintain glutathione synthesis, as highlighted by Ebuehi *et al.* (2023), further explains its protective effect on SOD and CAT activities.

The total antioxidant capacity (TAC), a measure of the cumulative action of all antioxidants, followed a similar trend. The significant decline in TAC in the IVM group corroborates the findings of Ekundayo *et al.* (2023), who reported that ivermectin treatment in rabbits significantly reduced serum TAC, indicating a depletion of both enzymatic and non-enzymatic antioxidant pools. In contrast, the significant elevation of TAC in the IVM + F.A group, surpassing even the control group, suggests a synergistic or additive antioxidant effect of folic acid. This is consistent with the results of Hassan *et al.* (2023), who found that folic acid supplementation significantly improved serum TAC in rabbits challenged with methotrexate-induced oxidative stress, due to its role in facilitating methionine synthase activity and subsequent synthesis of S-adenosylmethionine (SAMe), a critical methyl donor involved in antioxidant defence mechanisms. Furthermore, Adegoke *et al.* (2023) demonstrated that folic acid, through its involvement in homocysteine metabolism, reduces oxidative stress by preventing homocysteine-induced ROS generation, which explains the robust antioxidant response observed in the present study.

Malondialdehyde (MDA), a reliable biomarker of lipid peroxidation and cellular membrane damage, was significantly elevated in the IVM group, confirming that ivermectin-induced oxidative stress resulted in extensive lipid peroxidation. This finding is in strong agreement with the observations of Ogbuewu *et al.* (2023), who reported that ivermectin administration in female rabbits caused a significant increase in serum and tissue MDA levels, indicating severe oxidative membrane injury. Conversely, the significant reduction in MDA levels in the IVM + F.A group demonstrates the protective effect of folic acid against oxidative membrane damage. The findings also mirror those of Chen *et al.* (2023), who reported that folic acid's antioxidant properties effectively suppressed MDA formation in the liver and reproductive tissues of female animals exposed to oxidative stress, supporting its role in protecting against drug-induced oxidative damage.

The complete reversal of IVM-induced oxidative damage by folic acid suggests a potent cytoprotective role in female rabbits. Folic acid's antioxidant and anti-inflammatory properties are crucial for mitigating drug-induced toxicity in female animal models, particularly in the context of reproductive health. The results from the current study confirm that while ivermectin alone compromises the oxidative balance by suppressing SOD and CAT activities and elevating MDA, its co-administration with folic acid effectively restores redox homeostasis. This underscores the potential of folic acid as a functional nutritional intervention to protect against pharmaceutical-induced oxidative stress in female rabbits, which may have significant implications for maintaining reproductive performance and overall health in treated animals.

CONCLUSION

The study concludes that ivermectin has a negative impact on oxidative stress biomarkers in female rabbits by suppressing enzymatic and non-enzymatic antioxidant systems and promoting lipid peroxidation. In contrast, folic acid supplementation exhibits a strong protective effect by restoring antioxidant enzyme activities and reducing oxidative damage. Therefore, folic acid plays a crucial cytoprotective role and can be considered an effective nutritional intervention for mitigating ivermectin-induced oxidative stress. This protective effect is particularly important for maintaining physiological stability and potentially improving reproductive health in female rabbits exposed to pharmaceutical treatments.

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CONFLICT OF INTEREST

None

REFERENCES

1. Adegoke, A. O., Olatunji, S. Y., & Adekunle, A. S. (2023). Role of folic acid in homocysteine metabolism and oxidative stress reduction in animal models. *Journal of Animal Physiology and Animal Nutrition*, *107*(2), 456–465.
2. Adeyemi, O. S., & Akinlolu, A. A. (2023). Sub-chronic ivermectin exposure alters antioxidant gene expression in female Wistar rats. *Toxicology Reports*, *10*, 112–120
3. Chen, X., Liu, Y., Zhang, H., & Wang, J. (2023). Protective effects of folic acid against oxidative stress in liver and reproductive tissues of female animals. *Antioxidants*, *12*(4), 789
4. Ebuehi, O. A. T., Ajayi, I. O., & Ogunlade, B. (2023). Folic acid and glutathione metabolism: Implications for antioxidant defense in mammals. *Biochemistry Research International*, *2023*, 1–10.
5. El-Shobokshy, S. A., Abo-Samaha, M. I., Abd El-Rheem, S. M., Sahwan, F. M., Wirtu, G., Soltan, M. A. K., & Emam, M. (2022). Dietary supplementation of nano-selenium eliminates the negative effects of long-term ivermectin injection on growth and reproductive performance of female rabbits. *Journal of advanced veterinary and animal research*, *9*(1), 128.
6. Ekundayo, O. J., Oladipo, O. O., & Sanni, K. B. (2023). Ivermectin-induced oxidative stress and

*Corresponding Author: **Baba-Onoja O.M.**

depletion of antioxidant capacity in rabbits. *Veterinary and Animal Science*, *20*, 100285.

7. Hassan, M. A., El-Sayed, N. A., & Abd El-Hack, M. E. (2023). Folic acid supplementation improves antioxidant status in rabbits under oxidative stress conditions. *World Rabbit Science*, *31*(1), 25–34.
8. Ogbuewu, I. P., Uchegbu, M. C., & Okoli, I. C. (2023). Oxidative stress biomarkers in rabbits following ivermectin administration. *Comparative Clinical Pathology*, *32*(3), 789–798.
9. Saud, M. A., Khamees Abed, S., Shahooth, M. A., Jadaan, M., & Emad Abood, A. (2026). Physiological and Biochemical Responses to Repeated Administration of Ivermectin in Rabbits. *F1000Research*, *15*, 225.
10. Usman, S. S., Dahiru, M., Abdullahi, B., Abdullahi, S. B., Maigari, U. M., & Uba, A. I. (2019). Status of malondialdehyde, catalase and superoxide dismutase levels/activities in schoolchildren with iron deficiency and iron-deficiency anemia of Kashere and its environs in Gombe State, Nigeria. *Heliyon*, *5*(8).

*Corresponding Author: **Baba-Onoja O.M.**