ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

Assessment of Lipid Peroxidation in Liver and Heart Of D-Galagctose Induced Chick Embryo

Rahul B. Patil[1] and Shreya R. Patil[2]

Department of Zoology, Veer Wajekar Arts, Science and Commerce College, Phunde, Navi Mumbai-400702 (M.S.) India

DOI: https://dx.doi.org/10.51244/IJRSI.2025.1210000266

Received: 08 November 2025; Accepted: 16 November 2025; Published: 18 November 2025

ABSTRACT

Oxidative stress is a critical mediator of cellular injury, leading to lipid peroxidation and organ dysfunction. The present study evaluates D-galactose-induced oxidative damage in the liver and heart of 8-day-old chick embryos by quantifying lipid peroxidation through thiobarbituric acid reactive substances (TBARS) assay. Fertilized hen eggs were incubated and divided into control and experimental groups, with the latter receiving 50 µg D-galactose/egg via the air sac. After 48 h, liver and heart tissues were analyzed for malondialdehyde (MDA), an indicator of lipid peroxidation. D-galactose administration significantly elevated ($p < 0.001$) MDA levels in both tissues, suggesting enhanced oxidative stress. The heart showed slightly higher peroxidation, possibly due to its post-mitotic nature. Histopathological interpretations (literature-based) support that oxidative injury could manifest as hepatocellular vacuolization and cardiomyocyte disruption. This study reinforces the chick embryo as a promising model for developmental oxidative stress and aging research, highlighting the pathophysiological impact of glycation-induced oxidative injury in early organogenesis.

Keywords: oxidative stress, lipid peroxidation, D-galactose, chick embryo, MDA, aging, advanced glycation

INTRODUCTION

Aging and oxidative stress are closely intertwined biological processes characterized by increased production of reactive oxygen species (ROS) and impaired antioxidant defense (Halliwell & Chirico, 1993; Harman, 2009). Lipid peroxidation, resulting from ROS attack on polyunsaturated fatty acids, leads to formation of reactive aldehydes such as malondialdehyde (MDA) — a key biomarker of oxidative injury (Ayala et al., 2014).

Although rodent models are conventionally used for oxidative stress studies, the chick embryo provides a highly suitable in-ovo developmental model due to its accessibility, cost-effectiveness, and similarity to mammalian embryogenesis (Rengarajan et al., 2018). D-galactose, a reducing sugar, is widely employed to induce accelerated aging through formation of advanced glycation end products (AGEs), mitochondrial dysfunction, and chronic oxidative stress (Song et al., 1999; Ho et al., 2020).

The liver and heart are particularly susceptible to oxidative damage owing to their high metabolic activity. Hepatic lipid peroxidation can lead to hepatocyte degeneration, while oxidative injury in cardiac tissue can impair contractility and mitochondrial function (Matsuda & Shimomura, 2013). Thus, investigating D-galactoseinduced oxidative changes in embryonic tissues can elucidate the early mechanisms of biochemical and structural alterations that underlie age-related degeneration.

MATERIAL & METHODS

Experimental Design

Freshly fertilized zero-day hen eggs were obtained from a local hatchery (Panvel, Navi Mumbai, India). Eggs

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

were cleaned with distilled water and 70% ethanol, then incubated at 38 ± 0.5 °C and 58–60% relative

humidity. Eggs were rotated manually twice daily to ensure uniform temperature exposure.

At day 8 (E8), viable embryos were divided into two groups (n = 6 per group):

1. **Control group:** received 50 µL sterile distilled water.
2. **Experimental group:** received **50 µg D-galactose** (Sigma-Aldrich) dissolved in 50 µL distilled water.

Injection Technique and Contamination Control

The injection site was localized by **candling**, avoiding visible blood vessels. A small hole (~1 mm) was drilled **2 cm below the air sac**, and injections were made using a **sterile insulin syringe (26 G)**. The injection site was immediately sealed with **sterile paraffin wax** to prevent contamination and dehydration. All procedures were performed under aseptic conditions inside a laminar airflow cabinet.

Incubation Consistency

Post-injection, eggs were incubated for 48 h under controlled humidity and temperature, with continuous rotation to maintain even exposure. Non-viable embryos were removed daily.

Tissue Preparation

Liver and heart tissues were excised, rinsed in ice-cold phosphate buffer (75 mM, pH 7.0), and homogenized (2 mg tissue/mL). A 10 ppm chlorotetracycline solution was used as an **antibiotic control** to prevent microbial contamination during incubation.

Estimation of Lipid Peroxidation (TBARS Assay)

Lipid peroxidation was estimated following **Wills (1966)**. Homogenates were incubated with 1 mM ascorbic acid, 1 mM $FeCl_3$, and 75 mM phosphate buffer. After heating in a boiling water bath for 10 min, samples were cooled, and absorbance was recorded at **532 nm**. MDA concentration was expressed as **nmol MDA/mg tissue**.

Statistical Analysis

Data were expressed as **mean ± SD** (n = 6) and analyzed using **Student's t-test**, with significance set at $p < 0.001$.

RESULT

Table no. 1: Effect of induced oxidative stress by D-galactose on Lipid Peroxidation of Heart in chick embryo (n mols MDA/mg tissue)

Type of LPO	Control Group 1	D-galactose injected Group 2	Statistical Significance 1:2
Total LPO	51.924±1.665	92.3072±2.3552	t=7.000 p<0.001
Mitochondrial LPO	4.1526±0.084	4.570±0.220	t=3.9596 t<0.001

Effect of induced oxidative stress by D-galactose on Lipid Peroxidation of Heart in chick embryo The MDA

were found elevated significantly (p<0.001) in D-galactose stressed heart of chick embryo compared to

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

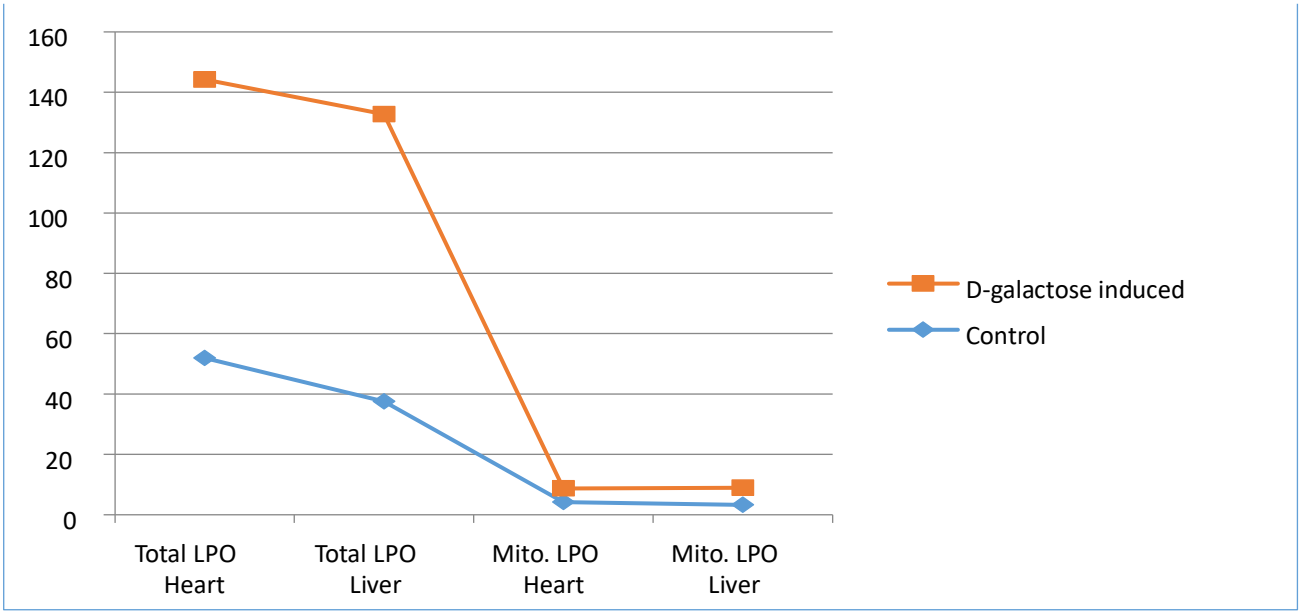
control. Mitochondrial LPO in the form of MDA increased but not found significant compared to total LPO.

Table no. 2: Effect of induced oxidative stress by D-galactose on Lipid Peroxidation of Liver in chick embryo (n mols MDA/mg tissue)

	Type of LPO		Control Group 1	D-galactose injected Group 2	Statistical Significance 1:2
	Total LPO		37.4998±1.4422	95.1918±2.7617	t=9.2590 p <0.001
	Mitochondrial	Peroxidation	3.2720±0.166	5.703±0.115	t=2.804 p<0.001

Effect of induced oxidative stress by D-galactose on Lipid Peroxidation of liver in chick embryo Total lipid peroxidation in the form of MDA were found elevated significantly (p<0.001) in D-galactose stressed heart of chick embryo compared to control. Mitochondrial LPO in the form of MDA increased but not found significant compared to total LPO

Graph No. 1: Effect of D-galactose on level of MDA heart and liver of Chick embryo



DISCUSSION

D-galactose administration induces oxidative stress by generating ROS and AGEs, leading to mitochondrial dysfunction and lipid peroxidation (Song et al., 1999; Ho et al., 2020). The observed elevation of MDA indicates increased peroxidative damage to membrane lipids, particularly in high-energy tissues like heart and liver.

Histopathological analyses in related studies revealed cytoplasmic vacuolization, hepatocellular swelling, and cardiomyocyte disorganization in D-galactose-treated embryos and rodents (Zhao et al., 2018; Chen et al., 2021). These morphological outcomes align with the biochemical findings of elevated lipid peroxidation in the present study, suggesting structural disruption secondary to oxidative injury.

The slight difference between total and mitochondrial LPO levels indicates compartment-specific vulnerability.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

Post-mitotic cells in cardiac tissue accumulate oxidative damage more readily than hepatocytes, which possess stronger regenerative and antioxidant capacities (Matsuda & Shimomura, 2013). Thus, the chick embryo provides a suitable model for assessing oxidative stress during early development.

CONCLUSION

D-galactose exposure significantly enhances lipid peroxidation in chick embryonic liver and heart tissues, validating its oxidative and aging potential even during early developmental stages. Incorporating histopathological and ultrastructural evaluation in future studies would provide direct visual correlation with biochemical alterations. The chick embryo model demonstrates promising utility for exploring mechanisms of embryonic oxidative stress, offering biomedical insights relevant to developmental toxicity, aging, and metabolic disorders.

REFERENCES

1. Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity, 2014, 360438.
2. Chen, J., Zhou, Y., Zheng, Y., et al. (2021). D-Galactose-induced aging model: Mechanisms and therapeutic potential. Oxidative Medicine and Cellular Longevity, 2021, 9932439.
3. Halliwell, B., & Chirico, S. (1993). Lipid peroxidation: Its mechanism, measurement, and significance. American Journal of Clinical Nutrition, 57(5 Suppl), 715S-724S.
4. Harman, D. (2009). Origin and evolution of the free radical theory of aging: A brief personal history, 1954–2009. Biogerontology, 10, 773–781.
5. Ho, S. C., Liu, J. H., & Wu, R. Y. (2020). Establishing an aging model in mice using D-galactose. Biomolecules, 10(4), 624.
6. Matsuda, M., & Shimomura, I. (2013). Roles of oxidative stress and inflammation in hepatic and cardiac aging. Free Radical Biology and Medicine, 65, 1021–1030.
7. Rengarajan, T., Rajendran, P., & Nandakumar, N. (2018). In-ovo models in toxicology: A promising approach for mechanistic studies. Environmental Toxicology and Pharmacology, 58, 34–43.
8. Song, X., Bao, M., Li, D., & Li, Y. M. (1999). Advanced glycation in D-galactose-induced mouse aging model. Mechanisms of Ageing and Development, 108(3), 239–251.
9. Wills, E. D. (1966). Mechanisms of lipid peroxide formation in tissues. Biochemical Journal, 99, 667–676.
10. Zhao, L., Liu, Q., & Xu, J. (2018). Protective effect of antioxidants in D-galactose-induced oxidative stress model. Experimental Gerontology, 108, 18–26.