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Research Paper

Ameliorative Potential of Luteolin Against D-galactose-Induced Hepatotoxicity and Metabolic Alterations in Aging Mice

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ABSTRACT

Background: Aging is a gradual decline in physiological functions that affect various organs of the body, including the liver. D-galactose is a reducing sugar used to mimic aging in animal models when administered in high doses. The study investigated the ameliorative potential of luteolin against D-galactose-induced hepatotoxicity and metabolic alterations in aging mice.

Methods: Twenty-five male mice were randomly divided into five groups (n=5). D-galactose (200 mg/kg) was administered subcutaneously for 56 days to induce aging, while luteolin (10 or 20 mg/kg) was administered orally to the intervention groups from day 29 to day 56. After administration, fasting blood glucose was assessed via the tail vein. Blood samples were collected to evaluate serum glycated hemoglobin, lipid profile - total cholesterol (Tcho), low-density lipoprotein (LDL-c), triglyceride (TG) and high-density lipoprotein (HDL-c) levels, and liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The livers were harvested for histological examination.

Results: D-galactose did not cause significant changes in glycemic indices in the treatment groups when compared to the control. Luteolin significantly (p<0.05) reversed D-galactose-induced lipid alterations with decreases in Tcho, LDL-c and TG levels, and an increase in HDL-c level.

The ALT, AST, and ALP levels significantly decreased (p<0.05) in the luteolin intervention groups compared to the untreated D-galactose group. Liver histology showed that luteolin treatment preserved hepatic architecture and protected the liver against injuries.

Conclusion: These findings suggest that luteolin exhibits natural ameliorative potential capable of reversing dyslipidemia, hepatic dysfunction, and metabolic alterations associated with aging.

Keywords: Aging, Galactose, Hepatotoxicity, Luteolin, Metabolism

Introduction

Aging is a complex biological process characterized by progressive tissue deterioration, gradual loss of physiological functions, compromised immunity and increased susceptibility to age-related diseases, which may ultimately result in death. Although chronic inflammation and oxidative stress have been widely associated with a potential risk factor in the development of age-associated diseases [1,2], metabolic dysregulation is a key contributor to the aging process and its related pathologies [3-5].

The liver is the central organ of metabolism, responsible for maintaining whole-body homeostasis through the regulation of energy metabolism, endobiotic and xenobiotic clearance, and molecular biosynthesis [6]. Liver aging is associated with gradual, structural, and functional alterations of the liver integrity leading to a decrease in the number of mitochondria [7], loss of hepatocytes responsible for the majority of hepatic functions [8], decline in liver functions, and metabolic dysfunction that negatively impact other systems, including the cardiovascular and nervous systems. Age-related conditions, such as insulin resistance, diabetes mellitus, non-alcoholic fatty liver disease, dyslipidemia, and impaired glucose tolerance, have been linked to metabolic dysfunction associated with liver aging [7,9,10].

D-galactose is a reducing sugar that mimics the effects of aging in animal models when administered in high doses. Excess systemic D-galactose administration contributes to the increased formation of reactive oxygen species (ROS). Elevated levels of ROS that exceed the antioxidant defense capacity induce oxidative stress, promoting the formation of advanced glycation end products (AGEs), which in turn contribute to hepatocyte

damage [11,12]. The interaction between AGEs and their receptor, known as RAGE, is a common occurrence in various degenerative conditions related to aging. When RAGE is excessively expressed, it can trigger the activation of nuclear factor-kappa B (NF-κB) along with other pro-inflammatory pathways, which are strongly linked to metabolic and age-associated disorders, most notably those involving the liver and pancreas [13]. This cascade of events often results in widespread physiological disturbances, including impaired liver function, disrupted lipid metabolism, and imbalanced blood glucose regulation.

Flavonoids have become a focal point in scientific studies because of their significant antioxidant and therapeutic roles within living systems [14,15]. Luteolin stands out as a naturally derived flavonoid recognized for its strong antioxidant capacity, anti-inflammatory action, ability to promote programmed cell death, and potential role in cancer prevention [16,17]. It is found in various fruits, vegetables, and medicinal herbs, and is well known for scavenging free radicals and protecting cells from damage associated with ROS [18].

The anti-aging properties of luteolin have recently been reported in rat skin models [19]. It has also been found to attenuate liver injury and counteract liver damage via its antioxidant and anti-inflammatory properties [20,21]. The current study evaluated the ability of luteolin to attenuate age-related changes in the morphology of the liver, blood glucose, glycated hemoglobin (HbA1c), serum liver enzymes, and lipid profile levels in D-galactose-induced aging rats.

Materials and Methods

Reagents and Drug Preparations

D-galactose ($C_6H_{12}O_6$) and Luteolin ($C_{15}H_{10}O_6$) were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Dimethyl sulphoxide (DMSO) and 0.9% normal saline were commercially purchased. The reagents used were of reagent grade and were also commercially purchased. D-galactose (200 mg/kg) was dissolved in normal saline [22]. Luteolin 10 and 20 mg/kg, respectively, were dissolved in 1% Dimethyl sulfoxide (DMSO). The doses of luteolin were selected based on previous reports demonstrating their efficacy in mitigating oxidative stress and metabolic alterations [23].

Experimental Animals

Twenty-five male mice (weighing between 20 and 26 g) were used in this study. The animals were purchased from the college animal house. They were kept in clean, metallic gauze cages with sawdust as bedding, placed in a well-ventilated room with optimum conditions (temperature 25±5°C, humidity 45-50%), and subjected to a 12/12-h light/dark cycle. The animals were acclimated for one week before stress induction and were fed rat chow and water *ad libitum*. The Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, granted ethical approval for this study (approval No.: ***285PHY1424***). All procedures involving the experimental animals used were carried out in strict accordance with the Committee's regulations and conformed to the animal care guidelines established by the United Kingdom.

Experimental Design

Twenty-five male mice (20-26 g) were used in this study. The animals were randomly assigned to five experimental groups with five rats per cage. The groups were:

- Group 1- Control (CT): Received 0.2 mL normal saline subcutaneously from day 1 to day 56, and 0.2 mL distilled water orally (*p.o*) from day 29 to day 56.
- Group 2- Luteolin (LU): Received phosphate buffer saline (PBS) subcutaneously from day 1 to day 56, and Luteolin 20 mg/kg (*p.o*) from day 29 to day 56.
- Group 3- D-galactose (DG): Received D-galactose (200 mg/kg) subcutaneously from day 1 to day 56, and vehicle (*p.o*) from day 29 to day 56.
- Group 4- D-galactose plus Luteolin (DG+L1): Received D-galactose (200 mg/kg) subcutaneously from day 1 to day 56, and Luteolin 10 mg/kg (*p.o*) from day 29 to day 56.
- Group 5- D-galactose plus Luteolin (DG+L2): Received D-galactose (200 mg/kg) subcutaneously from day 1 to day 56, and Luteolin 20 mg/kg (*p.o*) from day 29 to day 56.

Determination of Fasting Blood Glucose

A fasting blood glucose test was carried out on the experimental animals on the day of sacrifice. Blood samples were collected from the tail vein after an overnight fast. The level of fasting blood glucose was measured using a commercial glucose analyzer, Fine Test Auto-Coding Premium (Osang Healthcare Co., Ltd., Korea) glucometer.

Blood Collection and Biochemical Assays

At the end of the 8-week administration period, the animals were anesthetized with ketamine (80 mg/kg), and blood samples were collected via cardiac puncture into ethylene diamine tetra-acetic acid (EDTA) sample bottles for HbA1c assessment. Another portion was put into plain sample tubes. The blood in the plain tubes was centrifuged at 3,000 rpm for 10 min, and the serum was separated for biochemical assay of lipid profile and liver enzymes.

Estimation of Blood Glycated Hemoglobin (HbA1c) Level

The blood HbA1c level was estimated using a commercial reagent kit (Biodiagnostic, Egypt) according to the manufacturer's instructions.

Estimation of Lipid Profile

The lipid profile was enzymatically determined using

colorimetric methods with an Aggape assay kit (India). High-density lipoprotein cholesterol (HDL-c) was measured as described by Rifai and Warnick [24], total cholesterol (Tcho) was measured as described by Allain [25], triglyceride levels (TG) were measured as described by Bucolo and David [26], and low-density lipoprotein cholesterol (LDL-c) was done as described by Crouse *et al.* [27].

Estimation of Liver Enzymes

Alanine transaminase (ALT) and Aspartate aminotransferase (AST) activities were estimated using the Agappe kits (India), based on methods described by Reitman and Frankel [28]. Alkaline phosphatase (ALP) activity was determined using the Agappe kit (India), based on methods described by Kind and King [29].

Histological Study

At the end of the administration, liver tissues were harvested from euthanized animals and immediately fixed in 10% formaldehyde saline for 48 h. They were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a rotary microtome, mounted on albumenized slides, and stained with hematoxylin and eosin (H&E) using standard protocols. Stained slides were examined under a light microscope at magnifications of ×100 and ×400. The photomicrographs were captured for documentation and comparison across groups.

Statistical Analysis

Data were presented as means ± standard error of the means (SEM). GraphPad Prism (8.0) statistical software was used for statistical analyses. Comparison between different groups was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A $p < 0.05$ was considered statistically significant.

Results

Comparison of Blood Glucose and Glycated Hemoglobin Levels in D-galactose-induced Mice Treated with Luteolin (Table 1):

As shown in Table 1, blood glucose and glycated hemoglobin (HbA1c) levels showed no significant difference across the groups.

Comparison of Lipid Profile Levels in D-galactose-induced Mice Treated with Luteolin (Table 1):

The total cholesterol (Tcho) level significantly increased ($p<0.01$) in the DG group compared to the control group. However, it decreased significantly ($p<0.01$) in the DG+L2 group compared with the DG group (Table 1).

The low-density lipoprotein cholesterol (LDL-c) level significantly increased ($p<0.001$) in the DG group compared to the control group. However, it decreased significantly ($p<0.05$ and $p<0.01$) in the DG+L1 and DG+L2 groups, respectively, compared with the DG group (Table 1).

Triglyceride (TG) level significantly increased ($p<0.01$) in the DG group compared to the control group. However, it decreased significantly ($p<0.05$ and $p<0.01$) in the DG+L1 and DG+L2 groups, respectively, compared with the DG group (Table 1).

The high-density lipoprotein cholesterol (HDL-c) level significantly decreased ($p<0.001$) in the DG group compared to the control group. However, HDL-c levels increased significantly ($p<0.01$) in DG+L1 and DG+L2 groups, compared with the DG group (Table 1).

Table 1. Blood glucose (BG), glycated hemoglobin (HbA1c), and Lipid profile levels of D-galactose-induced mice treated with luteolin

Parameters	CT	LU	DG	DG+L1	DG+L2
BG (mg/dL)	3.86 ± 0.07	3.81 ± 0.25	4.54 ± 0.41	3.75 ± 0.15	4.00 ± 0.16
HbA1c (ng/l)	3.86 ± 0.08	4.15 ± 0.36	4.00 ± 0.27	3.75 ± 0.15	3.60 ± 0.24
Tcho (mg/dL)	117.30 ± 1.94	121.90 ± 2.14	131.40 ± 1.03**	125.60 ± 1.70	118.70 ± 3.70^{b}
LDL-c (mg/dL)	54.42 ± 1.75	57.02 ± 2.34	72.29 ± 3.27***	61.60 ± 1.66^{a}	59.05 ± 2.42^{b}
TG (mg/dL)	125.10 ± 1.21	123.80 ± 1.68	137.10 ± 1.39**	128.70 ± 2.16^{a}	126.00 ± 2.51^{b}
HDL-c (mg/dL)	39.24 ± 1.24	38.13 ± 0.79	26.60 ± 2.04***	36.97 ± 1.81^{b}	35.33 ± 1.80^{b}

Data are expressed as mean ± standard error of mean (SEM). CT = Control, LU = Luteolin (20 mg/kg), DG = D-galactose (200 mg/kg), DG+L1 = D-galactose plus Luteolin (10 mg/kg), and DG+L2 = D-galactose plus Luteolin (20 mg/kg). $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* control; $^{a}p < 0.05$, $^{b}p < 0.01$ *vs* DG.

Comparisons of Liver Enzyme Levels in D-galactose-induced Mice Treated with Luteolin

The aspartate aminotransferase (AST) significantly increased in the DG group ($p<0.001$) compared to the control group. The AST levels, however, decreased in the DG+L1 and DG+L2 groups, respectively ($p<0.01$ and $p<0.05$), compared with the DG group (Figure 1A).

The Alanine aminotransferase (ALT) levels exhibited a significant increase ($p<0.001$) in the DG group compared to the control group. However, it decreased significantly ($p<0.01$) in both DG+L1 and DG+L2 groups, compared with the DG group (Figure 1B).

The ALP increased significantly ($p<0.001$) in the DG group compared to the control group. The alkaline phosphatase (ALP) level, however, significantly decreased in the DG+L1 and DG+L2 groups, respectively ($p<0.05$ and $p<0.01$), compared with the DG group (Figure 1C).

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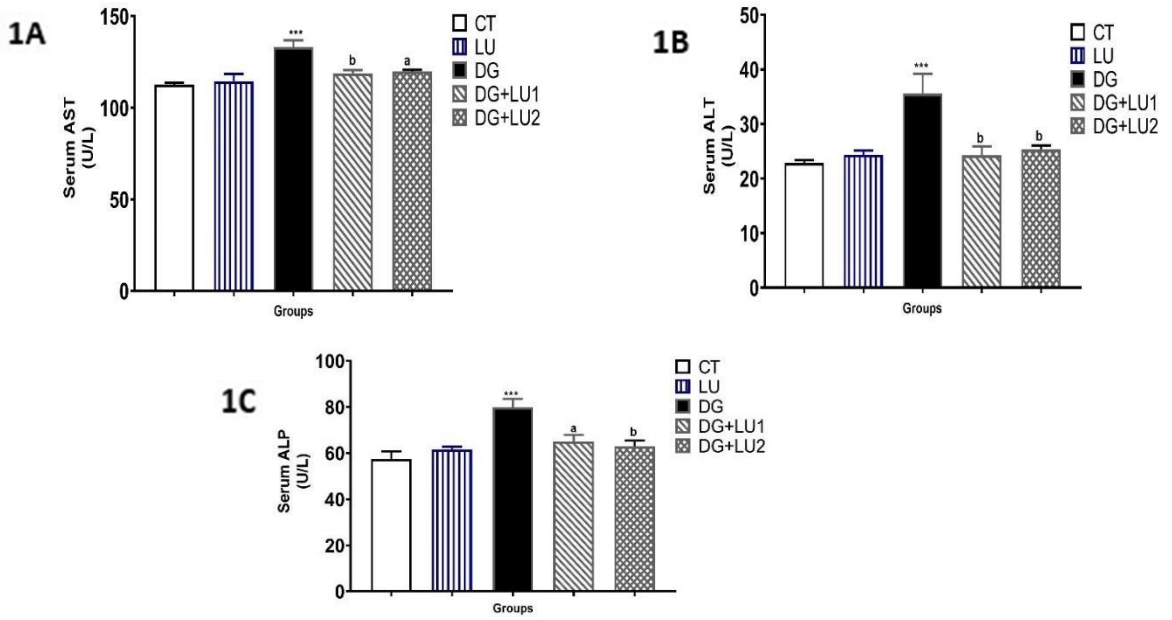

Figure 1. Comparison of liver enzymes (AST, ALT, ALP) in D-galactose-induced mice treated with Luteolin. Data are expressed as mean ± SEM. CT = Control, LU = Luteolin (20 mg/kg), DG = D-galactose (200 mg/kg), DG+L1 = D-galactose plus Luteolin (10 mg/kg), and DG+L2 = D-galactose plus Luteolin (20 mg/kg). $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* control; $^{a}p < 0.05$, $^{b}p < 0.01$ *vs* DG.

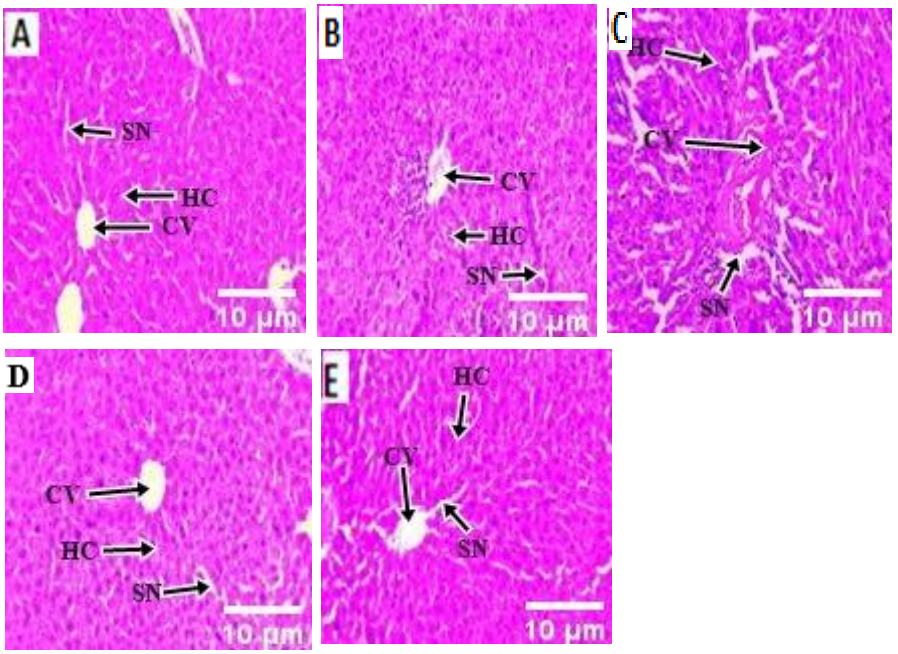

Figure 2. Photomicrograph of a liver section of D-galactose-induced mice treated with Luteolin, stained by Hematoxylin and Eosin (x 100 mag.). Groups: A= Control (CT), **B** = Luteolin (LU), **C** = D-galactose (DG), **D** = D-galactose plus Luteolin 10 mg/kg (DG+L1), **E** = D-galactose plus Luteolin 20 mg/kg (DG+L2).

Histological Findings

As shown in Figure 2, the cytoarchitecture of the liver tissue in group A appeared normal, with well-organized hepatocytes (HC) and sinusoids (SN) around the central vein (CV). Group B was similar to the control (A), with no significant changes. Group C showed disrupted cytoarchitecture, disorganized hepatocytes compared to the control group (A), with congested sinusoids and the presence of inflammation.

Group D showed partial improvement in the cytoarchitecture, with reduced inflammation, and enhanced hepatocytes and sinusoids compared to the D-galactose group (C), although not to the same extent as the control (A). Group E showed significant improvement in the cytoarchitecture, sinusoids, and hepatocytes when compared to the D-galactose group (C), and appeared almost similar to the control group (A).

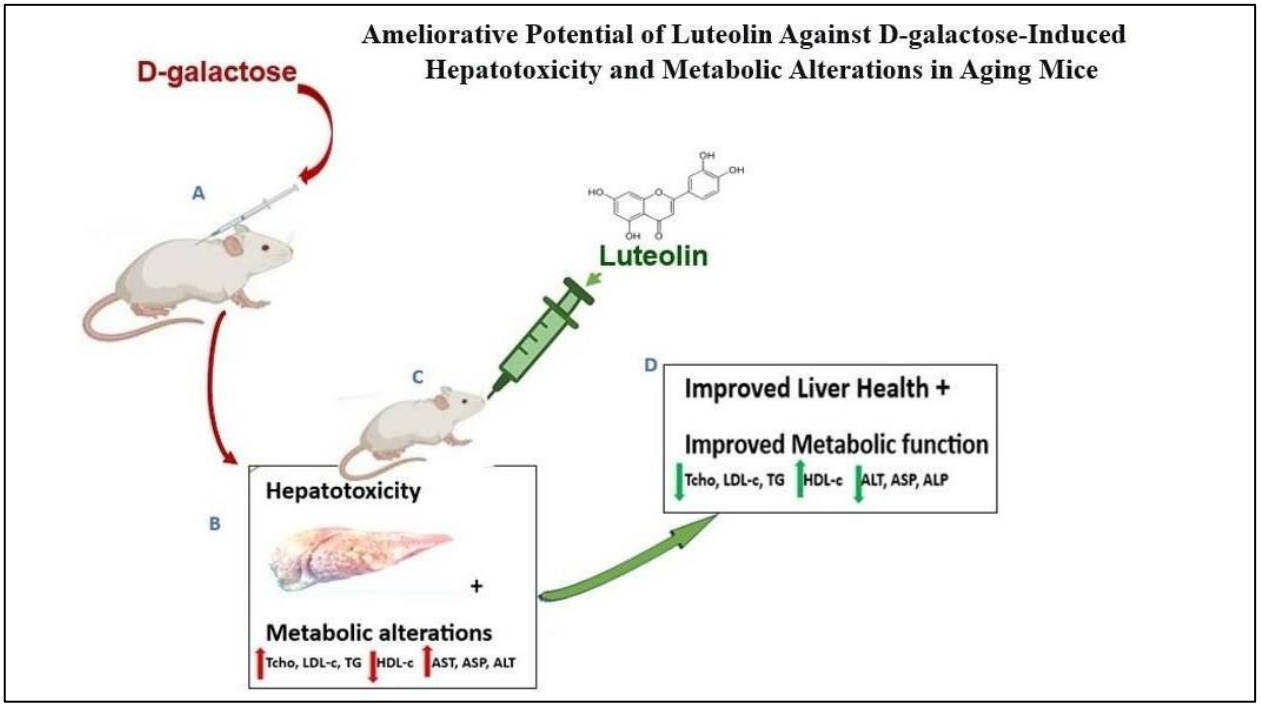

Figure 3. Graphical illustration of the Ameliorative Potential of Luteolin Against D-galactose-Induced Hepatotoxicity and Metabolic Alterations in Aging Mice. **A** = D-galactose-induced aging model (*subcutaneous administration*). **B** = Pathological state: Hepatotoxicity and Metabolic Alterations. **C** = Luteolin treatment (*oral administration*). **D** = Ameliorated State

Discussion

D-galactose is widely used to induce aging in animal models. When administered in high doses, it causes oxidative stress, inflammation, and accelerated aging by promoting the formation of AGEs and ROS, which can cause hepatocyte damage and metabolic alterations [11-13]. The present study investigated the effects of luteolin treatment on metabolic and liver alterations in mice, following an 8-week induction with D-galactose.

The effects of luteolin on blood glucose and glycated hemoglobin levels in mice induced with D-galactose were investigated in the current study to evaluate the potential of luteolin to reverse these glycemic parameters, previously reported to be altered following D-galactose induction [30]. In contrast to the findings by Du *et al.* [30], the current study found no significant alteration in fasting blood glucose and glycated hemoglobin levels across the D-galactose-induced groups compared to the control group. The lack of significant alterations in blood glucose and glycated hemoglobin levels in the present study suggests that D-galactose primarily induces aging via oxidative stress and inflammatory pathways rather than by disrupting glucose-insulin homeostasis. This result aligns with the findings by Gama *et al.* [31], who also reported no significant glycemic changes following D-galactose induction in rodents. Therefore, it is possible that pancreatic function remained largely intact, or that compensatory insulin mechanisms prevented glycemic disturbances.

The D-galactose aging model has been linked to alterations in lipid metabolism, resulting in metabolic disorders such as dyslipidemia, which causes as a significant risk factor in the development of cardiovascular diseases, hypertension, obesity, stroke, and dementia, which affects most older adults [32,33]. D-galactose induction triggered significant alterations in lipid profile levels, with increases in Tcho, LDL-c and TG, as well as a decrease in HDL-c level compared to the control group, thereby presenting a feature of age-related dyslipidemia. The D-galactose-induced dyslipidemia observed in this study aligns with the findings provided by Tarbiat *et al.,* who similarly reported dyslipidemia in Wistar rats following D-galactose administration [34]. Previous studies have suggested that age-related dyslipidemia may be linked to D-galactose-induced metabolic disturbances, which can interfere with the expression of adipogenic factors, such as peroxisome proliferator-activated receptors (PPARs), which are key regulators of lipid metabolism and energy homeostasis in the liver, brain, and adipose tissue [35,36].

In the present study, luteolin treatment significantly reversed the lipid profile alterations induced by D-galactose, evidenced by reductions in Tcho, LDL-c, and TG levels, alongside an increase in HDL-c levels. Although the underlying mechanisms were not directly assessed, luteolin has been reported in other studies to possess antioxidant activity and to modulate PPAR expression. These previously documented effects may partly explain the improvements in dyslipidemia observed in this study [37,38].

The optimal function of the liver is crucial for maintaining and regulating metabolic and homeostatic processes. A previous study showed the correlation between elevated liver enzyme levels and metabolic syndrome [39]. Elevated liver enzyme levels increase the

risk of developing chronic diseases associated with ageing [40]. In the current study, administration of a high dose of D-galactose caused significant increases in serum liver enzyme levels, including AST, ALT, and ALP, compared to the control group. Elevated levels of liver enzymes in the bloodstream often serve as indicators of liver dysfunction or hepatocyte damage [39,41].

Treatment with luteolin resulted in a significant reduction in the liver enzyme levels, indicating the potential of luteolin to improve hepatic function. The luteolin's ability to improve liver enzyme levels may be linked to the presence of its phenolic hydroxyl groups, which contribute to its antioxidant effects and its ability to reduce ROS-mediated hepatocyte injury. Similarly, some studies have indicated that its flavonoid-rich constituents provide several health benefits by modulating various biological activities, including cell signaling molecules, levels of enzymatic oxidants, and liver function enzymes [42-44].

The histological examination of the liver in the present study reinforced the biochemical findings, as seen in the photomicrograph of the liver. During the ageing process, liver function may decline due to increased susceptibility to injury and a higher risk of developing liver fibrosis and other structural changes. The photomicrograph of the liver histology of this study shows that, compared to the control group, D-galactose caused severe hepatic damage, evidenced by hepatocellular vacuolation, central vein congestion, inflammatory infiltration, and potential necrosis. These alterations resemble ageing-related liver pathology. A study revealed that D-galactose injection induced liver injury and dysfunction, which led to noticeable morphological alterations and elevated levels or activities of certain serum enzymes [45]. These histopathological changes in the liver were, however, ameliorated following treatment with luteolin. The luteolin treatment preserved hepatic architecture, reduced cellular degeneration, and minimized inflammation, with the 20 mg/kg dose providing a near-complete protection. The ability of luteolin to protect the liver from D-galactose-induced injury or damage, as seen in the histological findings of this study, may be linked to findings of other studies, which suggest that luteolin improves liver lesions through various mechanisms, including inhibiting inflammatory factors, reducing oxidative stress, regulating lipid balance, and slowing down excessive aggregation of extracellular matrix [46]. The overall mechanism of D-galactose-induced hepatotoxicity and the ameliorative role of luteolin is summarized in Figure 3. Luteolin thus exhibited significant hepato-protective and lipid-regulating effects in D-galactose-induced aging, as observed in the current study.

Conclusions

The findings indicate that luteolin ameliorated hepatic injury, improved serum lipid profiles, and preserved liver architecture. These findings suggest that luteolin exhibits natural ameliorative potential, effectively reversing dyslipidemia, hepatic dysfunction, and metabolic alterations associated with aging.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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Authors' Contributions

POO conceptualized the study. POO, JAB and SAB designed the work. POO and VKO contributed to the bench work. JAB, SAB, and COF provided expert advice and knowledge. POO drafted the manuscript. All authors read and contributed to the final manuscript, provided their inputs, and agreed with the submission.

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References

1. Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, et al. Molecular inflammation: Underpinnings of aging and age-related diseases. Ageing Res Rev. 2009;**8**(1):18-30. [**DOI**: 10.1016/j.arr.2008.07.002] [**PMID**: 18692159]
2. Odu P, Odu VK, Oyebanjo OT, Benneth BA, Onasanwo SA. Cognitive and neuroprotective effects of Vernonia amygdalina in scopolamine-induced memory impaired rats. Niger J Physiol Sci. 2024;**39**(2):233-40. [**DOI**: 10.54548/njps.v39i2.9] [**PMID**: 40156795]
3. Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent Pat Inflamm Allergy Drug Discov. 2009;**3**(1):73-80. [**DOI**: 10.2174/187221309787158371] [**PMID**: 19149749]
4. Çoban J, Betül-Kalaz E, Küçükgergin C, Aydın AF, Doğan-Ekici I, Doğru-Abbasoğlu S, et al. Blueberry treatment attenuates D-galactose-induced oxidative stress and tissue damage in rat liver. Geriatr Gerontol Int. 2014;**14**(2):490–7. [**DOI**: 10.1111/ggi.12096] [**PMID**: 23750655]
5. Omidkhoda SF, Mehria S, Heidaria S, Hosseinzadeh H. Protective effects of crocin against hepatic damages in D-galactose aging model in rats. Iran J Pharm Res. 2020;**19**(3):440–50. [**DOI**: 10.22037/ijpr.2019.15022.12825] [**PMID**: 33680043]
6. Rui L. Energy metabolism in the liver. Compr Physiol. 2014;**4**(1):177-97. [**DOI**: 10.1002/cphy.c130024] [**PMID**: 24692138]
7. Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nat. 2010;**469**(7328):102–6. [**DOI**: 10.1038/nature09603] [**PMID**: 21113150]
8. Hunt NJ, Kang SW, Lockwood GP, Le Couteur DG, Cogger VC. Hallmarks of aging in the liver. Comput Struct Biotechnol J. 2019;**17**:1151-61. [**DOI**: 10.1016/j.csbj.2019.07.021] [**PMID**: 31462971]
9. Niccoli T, Partridge L. Ageing as a risk factor for disease. Curr Biol. 2012;**22**(17):741-52. [**DOI**: 10.1016/j.cub.2012.07.024] [**PMID**: 22975005]
10. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;**153**(6):1194–217. [**DOI**: 10.1016/j.cell.2013.05.039] [**PMID**: 23746838]
11. Chen P, Chen F, Zhou B. Antioxidative, anti-inflammatory and anti-apoptotic effects of ellagic acid in liver and brain of rats treated by D-galactose. Sci Rep. 2018;**8**(1):1465. [**DOI**: 10.1038/s41598-018-19732-0] [**PMID**: 29362375]
12. Zhang Q, Li X, Cui X, Zuo P. D-galactose injured neurogenesis in

the hippocampus of adult mice. Neurol Res. 2005;**27**(5):552-6. [**DOI**: 10.1179/016164105x25126] [**PMID**: 15978184]
13. Ali T, Badshah H, Kim TH, Kim MO. Melatonin attenuates D-galactose-induced memory impairment, neuroinflammation and neurodegeneration via RAGE/NF-κB/JNK signaling pathway in aging mouse model. J Pineal Res. 2015;**58**(1):71–85. [**DOI**: 10.1111/jpi.12194] [**PMID**: 25401971]
14. Airoldi C, La Ferla B, D'Orazio G, Ciaramelli C, Palmioli A. Flavonoids in the treatment of Alzheimer's and other neurodegenerative diseases. Curr Med Chem. 2018;**25**(27):3228–46. [**DOI**: 10.2174/0929867324666170810142400] [**PMID**: 29424298]
15. Karak P. Biological activities of flavonoids: An overview. Int J Pharm Sci Res. 2019;**10**(4):1567–74. [**DOI**: 10.13040/IJPSR.0975-8232.10(4).1567-74]
16. Aziz N, Kim MY, Cho JY. Anti-inflammatory effects of luteolin: A review of in vitro, in vivo, and in silico studies. J Ethnopharmacol. 2018;**225**:342-58. [**DOI**: 10.1016/j.jep.2018.05.019] [**PMID**: 29801717]
17. Imran M, Rauf A, Abu-Izneid T, Nadeem M, Shariati MA, Khan IA, et al. Luteolin, a flavonoid, as an anticancer agent: A review. Biomed Pharmacother. 2019;**112**:108612. [**DOI**: 10.1016/j.biopha.2019.108612] [**PMID**: 30798142]
18. Mosa OF, Makkah KS. Protective impact of Luteolin on scopolamine induced-amnesia in wistar rats: An involvement of acetylcholinesterase, dopamine and lipid peroxidation. Life Sci J. 2021;**18**(3):4-11. [**DOI**: 10.7537/marslsj180321.02]
19. Mu J, Ma H, Chen H, Zhang X, Ye M. Luteolin prevents UVB-induced skin photoaging damage by modulating SIRT3/ROS/MAPK signaling: In vitro and in vivo studies. Front Pharmacol. 2021;**12**:728261. [**DOI**: 10.3389/fphar.2021.728261] [**PMID**: 34526903]
20. Yan Y, Jun C, Lu Y, Jiangmei S. Combination of metformin and luteolin synergistically protects against carbon tetrachloride-induced hepatotoxicity: Mechanism involves antioxidant, anti-inflammatory, antiapoptotic, and Nrf2/HO-1 signaling pathway. Biofactors. 2019;**45**(4):598–606. [**DOI**: 10.1002/biof.1511] [**PMID**: 31336028]
21. Zhang H, Tan X, Yang D, Lu J, Liu B, Baiyun R, et al. Dietary luteolin attenuates chronic liver injury induced by mercuric chloride via the Nrf2/NF-κB/p53 signaling pathway in rats. Oncotarget 2017;**8**(25):40982-93. [**DOI**: 10.18632/oncotarget.16684] [**PMID**: 28498799]
22. Cui X, Zuo P, Zhang Q, Li X, Hu Y, Long J, et al. Chronic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: Protective effects of R-alpha-lipoic acid. J Neurosci Res. 2006;**83**(8):1584–90. [**DOI**: 10.1002/jnr.20845] [**PMID**: 16555301]
23. Wang H, Wang H, Cheng H, Che Z. Ameliorating effect of luteolin on memory impairment in an Alzheimer's disease model. Mol Med Rep. 2016;**13**(5):4215–20. [**DOI**: 10.3892/mmr.2016.5044] [**PMID**: 27035793]
24. Rifai N, Warnick GR. Laboratory measurement of lipids, lipoproteins and apolipoproteins. Washington (DC): AACC Press. 1994:357. [**LINK**]
25. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;**20**(4):470-5. [**PMID**: 4818200]
26. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem. 1973;**19**(5):476-82. [**DOI**: 10.1093/clinchem/19.5.476] [**PMID**: 4703655]
27. Crouse JR, Parks JS, Schey HM, Kahl FR. Studies of low-density lipoprotein molecular weight in human beings with coronary artery disease. J Lipid Res. 1985;**26**(5):566-74. [**DOI**: 10.1016/s0022-2275(20)34343-1] [**PMID**: 4020295]
28. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;**28**(1):56-63. [**DOI**: 10.1093/ajcp/28.1.56] [**PMID**: 13458125]
29. Kind PR, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with Amino-antipyrine. J Clin Pathol. 1954;**7**(4):322-6. [**DOI**: 10.1136/jcp.7.4.322] [**PMID**: 13286357]
30. Du Z, Yang Y, Hu Y, Sun Y, Zhang S, Peng W, et al. A long-term high-fat diet increases oxidative stress, mitochondrial damage and apoptosis in the inner ear of D-galactose-induced aging rats. Hear Res. 2012;**287**(1–2):15-24. [**DOI**: 10.1016/j.heares.2012.04.012] [**PMID**: 22543089]
31. Gama AW, Akib R, Khaera U. The effect of giving D-galactose as an aging inducer on body weight, glucose levels and interleukin-6 levels in wistar rats. J Community Health Provis. 2025;**5**(1):1-3. [**DOI**: 10.55885/jchp.v5i1.556]
32. Gill TM, Hardy SE, Williams CS. Underestimation of disability in community-living older persons. J Am Geriatr Soc. 2002;**50**(9):1492-7. [**DOI**: 10.1046/j.1532-5415.2002.50403] [**PMID**: 12383145]
33. Odu PO, Ujah GA, Uket JM, Odu VK, Inwang UA. Costus afer leaves extract ameliorates stress-induced alterations in hematological and lipid parameters in wistar rats. Trop J Nat Prod Res. 2025;**9**(6):2821-6. [**DOI**: 10.26538/tjnpr/v9i6.63]
34. Tarbiat S, Aydın B, Ergün K, Mohseni AR. Assessing the impact of oleuropein on dyslipidemia in male rats subjected to D-galactose-induced aging: A preliminary study. Eur Chem Biotechnol J. 2024;**25**(2):17-26. [**DOI**: 10.62063/ecb-21]
35. Lin S, Beck AN, Finch BK, Hummer RA, Master RK. Trends in US older adult disability: exploring age, period, and cohort effects. Am J Public Health. 2012;**102**(11):2157-63. [**DOI**: 10.2105/AJPH.2011.300602] [**PMID**: 22994192]
36. Zhou Y, Xu Q, Dong Y, Zhu S, Song S, Sun N. Supplementation of mussel peptides reduces aging phenotype, lipid deposition and oxidative stress in D-galactose-induced aging mice. J Nutr Health Aging. 2017;**21**(10):1314-20. [DOI: 10.1007/s12603-016-0862-3] [**PMID**: 29188895]
37. Shehnaz SI, Roy A, Vijayaraghavan R, Sivanesan S. Luteolin mitigates diabetic dyslipidemia in rats by modulating ACAT-2, PPARα, SREBP-2 proteins, and oxidative stress. Appl Biochem Biotechnol. 2023;**195**(8):4893–914. [**DOI**: 10.1007/s12010-023-04544-4] [**PMID**: 37103741]
38. Dai X, Liang B, Sun Y. Luteolin ameliorates rat model of metabolic syndrome-induced cardiac injury by apoptosis suppression and autophagy promotion via NR4A2/p53 regulation. BMC Complement Med Ther. 2025;**25**(1):14. [**DOI**: 10.1186/s12906-025-04749-6] [**PMID**: 39833877]
39. Galal RM, Zaki HF, Seif MM, Agha AM. Potential protective effect of honey against paracetamol-induced hepatotoxicity. Arch Iran Med. 2012;15(11):674–80. [**PMID**: 23102243]
40. Villegas R, Xiang YB, Elasy T, Cai Q, Xu W, Li H, et al. Liver enzymes, type 2 diabetes, and metabolic syndrome in middle-aged, urban Chinese men. Metab Syndr Relat Disord. 2011;**9**(4):305-11. [**DOI**: 10.1089/met.2011.0016] [**PMID**: 21495862]
41. Anadón A, Castellano V, Martínez-Larrañaga MR. Biomarkers in drug safety evaluation. Biomark Toxicol. 2014:923-45. [**DOI**: 10.1016/B978-0-12-404630-6.00055-5]
42. Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. Curr Cancer Drug Targets. 2008;**8**(7):634-46. [**DOI**: 10.2174/156800908786241050] [**PMID**: 18991571]
43. Zhang Q, Yang J, Wang J. Modulatory effect of luteolin on redox homeostasis and inflammatory cytokines in a mouse model of liver cancer. Oncol Lett. 2016;**12**(6):4767–72. [**DOI**: 10.3892/ol.2016.5291] [**PMID**: 28101223]
44. Alharbi OA, Almatroudi A, Alrumaihi F, Alghafis SF, Alwanian WM, Rahmani AH. The potential role of luteolin, a flavonoid in cancer prevention and treatment. CyTA J Food. 2024;**22**(1): 2381714. [**DOI**: 10.1080/19476337.2024.2381714]
45. Lakshmi BVS, Sudhakar M, Prakash KS. Protective effect of selenium against aluminum chloride-induced Alzheimer's disease: Behavioral and biochemical alterations in rats. Biol Trace Elem Res. 2015;**165**(1):67–74. [**DOI**: 10.1007/s12011-015-0229-3] [**PMID**: 25613582]
46. Yao C, Dai S, Wang C, Fu K, Wu R, Zhao X, et al. Luteolin as a potential hepatoprotective drug: Molecular mechanisms and treatment strategies. Biomed Pharm. 2023;**167**:115464. [**DOI**: 10.1016/j.biopha.2023.115464] [**PMID**: 37713990]