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Ethylacetate Leaf Extract of *Adansonia digitata* Linn Mitigates Against Redox Imbalance and Inflammatory Disorders Associated with Ageing in Liver of Male Wistar Rat

Emmanuel Olagoke[1*], Olaniyi Adedosu[1], Gbadebo Adeleke[1], Omolara Babalola[1], Masaudat Adebayo[2], Mohammed Abdulrasak[3], Adetayo Akinboro[1], Oluwatosin Olagoke[1], Rafiu Musa[4]

[1]Department of Biochemistry, Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[2]Department of Biochemical Sciences, Federal Polytechnic, Ede, Nigeria.
[3]Department of Biochemistry, Federal University of Lafia, Lafia, Nigeria.
[4]Osun State University, Osogbo, Nigeria.
*Corresponding author: Emmanuel Olagoke, Department of Biochemistry, Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. E-mail: emmanuelolutunde34@gmail.com; Phone: +2347061366531, +2349062121016

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Abstract: Ageing is driven by oxidative stress, chronic inflammation, and impaired cellular repair. However, Adansonia digitata linn is employed as an anti-ageing remedy in West Africa with limited scientific basis. Hence, this study evaluated the potential of Adansonia digitata Ethylacetate Leaf Extract (ADELE) in mitigating against redox imbalance and inflammatory disorders associated with ageing in hepatic tissue of male Wistar rats. Ethylacetate extract of the powdered leaves of the plant was prepared by cold extraction. Sixty-three (63) male Wistar rats weighing an average of 110 g were randomly allocated into 9 groups of seven (7) rats each. Group I (control), Group II (ADELE-only 200 mg/kg body weight (bw)), Group III (Vitamin E-only 200 mg/kg bw), Group IV (D-galactose-only) were treated for 8 weeks, and pretreatment groups receiving graded doses of ADELE and/or Vitamin E 4 weeks prior to D-galactose administration for an additional 4 weeks: Group V (ADELE 100 mg/kg bw and D-gal), Group VI (ADELE 200 mg/kg bw and D-gal), Group VII (ADELE 400 mg/kg bw and D-gal), Group VIII (Vit. E 200 mg/kg bw and D-gal) and Group IX (ADELE 100 mg/kg bw and Vit. E 100 mg/kg bw and D-gal). ADELE was administered orally in 0.3 ml distilled water while D-galactose was administered intraperitoneally (IP) in 0.3 ml of 0.9% normal saline daily and animals were sacrificed after eight weeks. Liver indices [Alanine transaminase (ALT), Aspartate transaminase (AST) and Gamma-glutamyl transferase (GGT) activities and total bilirubin concentrations] in serum, antioxidant indices [Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) activities, Reduced glutathione (GSH), Malondialdehyde (MDA) and total protein concentrations], and inflammatory indices [Tumour Necrosis Factor (TNF-α) and Interleukin-6 (IL-6) concentrations] in liver homogenates were evaluated using standard biochemical methods. Data were analysed using GraphPad Prism 5 at ($P < 0.05$) level of significance. Results showed induction of ageing by D-galactose (Group IV) with significant increase ($P < 0.05$) in liver indices (ALT: 84.01 ± 3.86, AST: 95.17 ± 2.33, GGT: 29.49 ± 1.70 and total bilirubin: 50.70 ± 2.87), lipid peroxidation (MDA: 10.26 ± 0.89), inflammatory

* Corresponding Author: emmanuelolutunde34@gmail.com

cytokines (TNF-α: 6709 ± 833.00 and IL-6: 528.9 ± 35.31), and concomitant reductions in antioxidant enzyme activities (SOD: 0.69 ± 1.11, CAT: 4.07 ± 0.33 and GPx: 4.07 ± 0.13), as well as GSH: 3.60 ± 0.24 and hepatic total protein (TP): 27.04 ± 1.36, compared with controls (ALT: 70.09 ± 3.23, AST: 76.35 ± 2.51, GGT: 15.36 ± 0.52, total bilirubin: 38.74 ± 0.98, MDA: 3.84 ± 0.57, TNF-α: 1268 ± 82.66, IL-6: 345.6 ± 29.94, SOD: 1.43 ± 1.60, CAT: 5.19 ± 0.13, GPx: 6.07 ± 0.11, GSH: 4.18 ± 0.16, hepatic total protein: 38.00 ± 0.72). Pretreatment groups (V, VI, VII, VIII and IX) markedly attenuated D-galactose-induced alterations, restoring most biochemical parameters toward control levels, with some parameters showing dose-dependent activity. Histological examination revealed dose-dependent improvement in D-galactose-altered hepatic architecture toward control levels. ADELE exhibited antioxidant, anti-inflammatory, and tissue-protective potential; hence, its bioactive components may be employed in the treatment of oxidative stress- and inflammatory-related disorders as well as in the management of ageing.

Keywords: Adansonia digitata linn, antioxidant, anti-inflammatory, anti-ageing, D-galactose, redox imbalance.

1. Introduction

Ageing is a complex, multifactorial biological process characterised by the progressive decline in physiological function and increased susceptibility to chronic diseases. Among the key mechanisms implicated in ageing, oxidative stress and chronic low-grade inflammation — otherwise called “inflammagəing” (an elevated level of inflammatory markers in blood with susceptibility to chronic frailty, disability, and morbidity) — have emerged as key drivers of cellular and molecular deterioration (Ferrucci & Fabbri, 2018; Martemucci et al., 2022; Colombini et al., 2022). These interconnected processes influence multiple hallmarks of ageing, including genomic instability, mitochondrial dysfunction, cellular senescence, and altered intercellular communication. Oxidative stress occurs as a result of an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant defence systems to neutralise these reactive species. Under physiological conditions, ROS play important roles in cellular signalling; however, excessive ROS generation leads to oxidative damage of biomolecules such as proteins, lipids, and DNA (Leyane et al., 2022; Iakovou & Kourti, 2022). This accumulation of oxidative damage over time contributes to functional decline in tissues and organs, thereby accelerating the ageing process and predisposing individuals to age-related diseases such as neurodegeneration, cardiovascular disorders, and cancer (Martemucci et al., 2022).

Chronic inflammation is closely linked to oxidative stress; it is a persistent, low-grade immune response that develops with advancing age. Unlike acute inflammation, which is protective and resolves after injury or infection, chronic inflammation is sustained and contributes to tissue damage and dysfunction. Molecular pathways such as nuclear factor kappa B (NF-κB) signalling play a significant role in regulating inflammatory responses and have been strongly associated with ageing and immune system decline (Songkiatisak et al., 2022). The interaction between oxidative stress and inflammation is bidirectional: ROS can activate inflammatory signalling pathways, while inflammatory processes further increase ROS production, thereby creating a self-perpetuating cycle that exacerbates cellular damage. Moreover, mitochondrial dysfunction is a key link between oxidative stress and inflammation in ageing. Mitochondria are both a major source and target of ROS, and age-associated mitochondrial impairment leads to increased production of ROS and release of pro-inflammatory mediators (Chen et al., 2022). This contributes to the development of cellular senescence, characterised by irreversible growth arrest and secretion of pro-inflammatory cytokines, collectively referred to as the senescence-associated secretory phenotype (SASP). These

factors reinforce systemic inflammation and tissue degeneration. Furthermore, oxidative stress and inflammation influence epigenetic modifications and post-translational changes that regulate gene expression during ageing. Various alterations in these regulatory mechanisms further amplify the ageing phenotype and are implicated in various chronic degenerative conditions (Altanam et al., 2025). Emerging evidence also suggests that antioxidant systems, including glutathione and enzymatic defence systems, play crucial protective roles in mitigating oxidative damage and inflammatory responses, although their efficiency declines with age (Labarrere & Kassab, 2022). Oxidative stress and inflammation are deeply intertwined processes driving the biological ageing process and the onset of age-related diseases. Understanding their mechanisms will provide valuable insights into possible therapeutic interventions aimed at promoting healthy ageing and extending lifespan.

Adansonia digitata linn, otherwise called the baobab tree, is a long-lived, multipurpose plant species belonging to the family Malvaceae. It is widely distributed across the savannah regions of sub-Saharan Africa and is regarded as one of the most iconic and economically valuable indigenous trees on the African continent due to its nutritional, medicinal, and ecological significance (Silva et al., 2023). Historically, *Adansonia digitata* has been documented for over 4,000 years and was first scientifically described in the eighteenth century, highlighting its long-standing importance in traditional African systems of medicine and livelihood (Silva et al., 2023). Botanically, *Adansonia digitata* is characterised by a massive trunk capable of storing large amounts of water, which underlies its survival in arid and semi-arid climates. It thrives in regions experiencing prolonged dry seasons and is well adapted to harsh environmental conditions typical of tropical Africa (Abdulwaliyu et al., 2024). It produces large, ovoid fruits with a hard outer shell and a nutrient-dense pulp, which has gained global attention for its functional food properties and industrial applications (Silva et al., 2023).

Nutritionally, its fruit pulp is exceptionally rich in dietary fibre, vitamins (particularly vitamin C), and several essential minerals such as calcium, potassium, and magnesium. It also contains significant levels of bioactive compounds including flavonoids, polyphenols, and tannins, contributing to its antioxidant and therapeutic potential (Abdulwaliyu et al., 2024; Silva et al., 2023). These phytochemicals have been associated with various biological activities including antidiabetic, anti-inflammatory, and antimicrobial potential, thereby supporting the plant's relevance in both traditional and modern medicine (Abuljadayel, 2023). Ethnopharmacologically, various parts of *Adansonia digitata* — including the leaves, seeds, bark, and fruit pulp — are extensively used in African traditional medicine for the treatment and management of ailments such as fever, diarrhoea, cough, malaria, and microbial infections (Abuljadayel, 2023). Moreover, the fruit pulp is commonly processed into beverages, porridges, and sauces, contributing significantly to food security and rural economies (Silva et al., 2023). In recent years, the increasing demand for natural products has further increased the global relevance of baobab in the pharmaceutical, food, and cosmetic industries (Abdulwaliyu et al., 2024). *Adansonia digitata* represents a valuable plant resource with substantial nutritional, medicinal, and socio-economic importance. Its growing scientific interest underscores the need for further research into its phytochemical composition, pharmacological properties, and potential applications in addressing contemporary health challenges including ageing-associated disorders (Oladele et al., 2012).

Adansonia digitata linn fruits

Adansonia digitata linn leaves

2. Materials and Methods

2.1. Materials

The materials used were: measuring cylinders, spectrophotometer, micropipettes, centrifuge, water bath, serum bottles, dissecting set, disposable gloves, thumb pins, thermometer, stopwatch, 2 ml and 5 ml syringes and needles, pH meter, cotton wool, conical flasks, test-tubes and racks, spatula, beakers, refrigerator, sensitive weighing balance, homogeniser, dissecting ropes, dissecting board, retort stand, burette, funnel, Pasteur pipette, filter paper, separating funnel, and rotary evaporator.

2.2. Reagents and Salts

Distilled water, washing buffer, phosphate buffer, normal saline, carbonate buffer, Tris-glycine buffer, xanthine, copper salt, sodium hydroxide solution, ethylacetate, Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoate) (DTNB)], adrenaline, potassium dichromate ($K_2Cr_2O_7$), glacial acetic acid (GAA), Tris potassium chloride, hydrochloric acid (HCl), hydrogen peroxide, and corn oil. Laboratory kits for the quantitative in vivo determination of total protein, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), total bilirubin, gamma-glutamyl transferase (GGT), aspartate transaminase (AST), alanine transaminase (ALT), rat caspase-3, tumour necrosis factor (TNF-α), trichloroacetic acid (TCA), absolute methanol (99.9%), adrenaline, sulfanilamide, thiobarbituric acid (TBA), and reduced glutathione (GSH) were all purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used for the experiments were of analytical grade.

2.3. Identification of Plant Material

The plant *Adansonia digitata* linn leaves were obtained from a farmland in Ogbomoso, Oyo State, Nigeria, and were identified and authenticated in the Botany section of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, with Voucher Number (LHO 749) deposited. They were air-dried and blended into powdery form to increase the surface area and facilitate the extraction process.

2.4. Preparation of Ethylacetate Extract and D-galactose

Two hundred and fifty grams (250 g) of powdered leaves of *Adansonia digitata* linn were soaked in 2500 ml of ethylacetate, left for 3 days, filtered using muslin cloth, and the filtrate was concentrated on a rotary evaporator at 35°C and later dried on a water bath. The percentage yield of *Adansonia digitata* was 15.69%. The LD_{50} for oral administration of *Adansonia digitata* linn has been reported to be above 5000 mg/kg body weight by Rufai et al. (2006).

Chronic administration of D-galactose has been reported to induce accelerated ageing in experimental animals via a process that closely resembles natural ageing in humans (Ho et al., 2003). D-galactose was administered intraperitoneally (IP) at the dose of 60 mg/kg body weight in 0.3 ml of 0.9% normal saline daily for a period of four (4) weeks (Tayyaba et al., 2016; Yanar et al., 2011), after pretreatment with extract for four (4) weeks, while D-galactose-only, ADELE-only, and Vit. E-only groups were administered their respective treatments for a period of 8 weeks.

2.5. Experimental Design

The rats were divided into nine (9) groups of seven (7) rats each as follows:

Group I (Negative control): Rats were fed standard rat pellet and distilled water ad libitum for eight (8) weeks.

Group II (ADELE only 200 mg/kg bw): Rats were given 200 mg/kg bw of A. digitata in 0.3 ml distilled water only for eight (8) weeks.

Group III (Vit. E only 200 mg/kg bw): Rats were given 200 mg/kg bw of Vit. E in 0.3 ml corn oil for eight (8) weeks.

Group IV (D-galactose only – Positive control): Rats were given IP injection (0.3 ml) of D-galactose (60 mg/kg bw) for eight (8) weeks.

Group V (ADELE 100 mg/kg bw and D-Gal): Rats were pretreated with 100 mg/kg bw (0.3 ml) A. digitata via oral intubation for four (4) weeks followed by IP injection (0.3 ml) of D-galactose (60 mg/kg bw) for four (4) weeks.

Group VI (ADELE 200 mg/kg bw and D-Gal): Rats were pretreated with 200 mg/kg bw (0.3 ml) A. digitata via oral intubation for four (4) weeks followed by IP injection (0.3 ml) of D-galactose (60 mg/kg bw) for four (4) weeks.

Group VII (ADELE 400 mg/kg bw and D-Gal): Rats were pretreated with 400 mg/kg bw (0.3 ml) A. digitata via oral intubation for four (4) weeks followed by IP injection (0.3 ml) of D-galactose (60 mg/kg bw) for four (4) weeks.

Group VIII (Vit. E and D-Gal): Rats were pretreated with 200 mg/kg bw of Vit. E via oral intubation for four (4) weeks followed by IP injection (0.3 ml) of D-galactose (60 mg/kg bw) for four (4) weeks.

Group IX (Extract 100 mg/kg and Vit. E 100 mg/kg and D-gal): The rats were given 0.3 ml mixture of 100 mg/kg bw of A. digitata and 100 mg/kg bw of Vit. E for four (4) weeks followed by IP injection (0.3 ml) of D-galactose (60 mg/kg bw) for four (4) weeks.

2.6. Histopathological Method

Portions of liver, kidney, and heart specimens were collected from rats of all experimental groups at the end of the experimental period, fixed in 10% buffered formalin (pH 7.0), dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4–6 micron thickness sections were prepared and stained with haematoxylin and eosin (H&E) for histopathological study.

2.7. Methods of Assay

2.7.1 Determination of Serum Alanine Transaminase (ALT) Activity

The ALT activity was measured by the method of Reitman and Frankel (1957). ALT catalyses the transfer of an amino group from alanine to α-ketoglutarate, yielding pyruvate and glutamate; the pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH). The rate of decrease in NADH absorbance at 340 nm is directly proportional to ALT activity.

2.7.2 Determination of Serum Aspartate Transaminase (AST) Activity

Aspartate transaminase activity was determined by the method described by Karmen (1995). Aspartate transaminase catalyses amino group transfer from L-aspartate to α-ketoglutarate, forming oxaloacetate and L-glutamate. The oxaloacetate produced is reduced to malate via malate dehydrogenase (MDH), which oxidises NADH to NAD^+. The rate of decreased absorbance at 340 nm, as a result of the consumption of NADH, is directly proportional to the activity of AST.

2.7.3 Determination of Serum Gamma-glutamyl Transferase (GGT) Activity

Gamma-glutamyl transferase activity was measured by the method of Szasz (1969). GGT catalyses the transfer of the gamma-glutamyl group from gamma-glutamyl-4-nitroanilide to the acceptor glycylglycine to form 2-nitro-5-aminobenzoic acid, whose concentration is measured spectrophotometrically at 405 nm and is proportional to the catalytic concentration of GGT enzyme.

2.7.4 Determination of Serum Total Bilirubin Concentration

Total bilirubin concentration was determined by the method of Kaplan (1984). Bilirubin is converted to coloured azobilirubin by diazotised sulphanilic acid. Bilirubin glucuronide reacts directly in aqueous solution (direct bilirubin), while free bilirubin (plasma-bound) is first solubilised with dimethylsulphoxide (DMSO) to react (indirect bilirubin). In the determination of indirect bilirubin, the direct is also determined and the result corresponds to total bilirubin. The intensity of the colour formed is proportional to the bilirubin concentration, which absorbs at 540 nm.

2.7.5 Determination of Serum and Hepatic Total Protein Concentration

Total protein concentration was determined based on the method described by Dominiczak (1998). Protein reacts with Folin-Ciocalteu reagent to give a blue-coloured complex that absorbs at a wavelength of 500 nm.

2.7.6 Determination of Hepatic Superoxide Dismutase (SOD) Activity

The level of SOD activity was determined by the method of Misra and Fridovich (1975). The ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2 serves as the basis for this assay. The superoxide radical generated by the xanthine oxidase reaction causes the oxidation of epinephrine to adrenochrome, and the yield of adrenochrome produced per superoxide introduced increases with increasing pH and increasing concentration of epinephrine. This results in the autoxidation of epinephrine, with absorbance measured at 480 nm.

2.7.7 Determination of Hepatic Catalase (CAT) Activity

Catalase activity was measured by the method of Aebi (1984). Dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide, with the formation of perchromic acid as an unstable intermediate. The chromic acetate produced is measured at 570 nm. Since dichromate has no absorbance in this region, its presence in the assay mixture does not interfere with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split hydrogen peroxide for different periods of time; the reaction is stopped at a particular time by the addition of dichromate/acetic acid mixture, and the remaining hydrogen peroxide is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

2.7.8 Determination of Hepatic Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase (GPx) was determined by the method of Anderson et al. (1985). GPx catalyses the oxidation of reduced glutathione (GSH) to oxidised glutathione (GSSG) in the presence

of hydrogen peroxide. GSH and DTNB interact, forming 5-thio-2-nitrobenzoic acid (TNB), whose absorbance was spectrophotometrically estimated at 412 nm.

2.7.9 Determination of Hepatic Reduced Glutathione (GSH) Concentration

Reduced glutathione (GSH) was determined by the method of Andersen et al. (1997). Reduced glutathione estimation was based on the development of a relatively stable yellow colour that absorbs at 412 nm when 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) is added to sulphydryl compounds.

2.7.10 Determination of Lipid Peroxidation

Estimation of lipid peroxidation in hepatic tissue was based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA), forming an MDA-TBAR adduct that absorbs strongly at 532 nm, as described by Ohkawa et al. (1979).

2.7.11 Determination of Hepatic Tumour Necrosis Factor-α (TNF-α) Concentration

Hepatic TNF-α concentration was determined by the method described by Cedrone et al. (2020), which employed the enzyme-linked immunosorbent assay (ELISA) technique. The coated antibodies on the microplate wells bind to the TNF-α present in the sample. Detection antibody (biotin) was added, binding to a different site on the captured TNF-α molecule. Streptavidin-horseradish peroxidase was added, binding to the detection antibody and catalysing the colour-changing reaction; the developed blue colour intensity was measured at wavelength 450 nm. The colour developed is directly proportional to TNF-α concentration, which was quantified using a standard curve.

2.7.12 Determination of Hepatic Interleukin-6 (IL-6) Concentration

Hepatic IL-6 concentration was determined according to the method described by Helle et al. (1991), which employed the enzyme-linked immunosorbent assay (ELISA) technique. IL-6 cytokine selectively binds to a monoclonal antibody immobilised on a solid phase. Horseradish peroxidase was added to catalyse the reaction. A chromogenic substrate (tetramethylbenzidine (TMB)) was added to the wells, leading to the development of a blue colour. Hydrochloric acid (1 M) was added to each well to stop the reaction, turning the blue colour to yellow, which absorbs at wavelength 450 nm. The colour developed is directly proportional to IL-6 concentration, which was quantified using a standard curve.

2.8. Statistical Analysis

The results were analysed using GraphPad Prism 5 Software and reported as Mean ± SEM for seven rats per group. One-way analysis of variance (ANOVA) was used to determine the relative expression level of each group. The level of significance was taken at $p < 0.05$.

3.0 Results

(A) Liver Function Indices

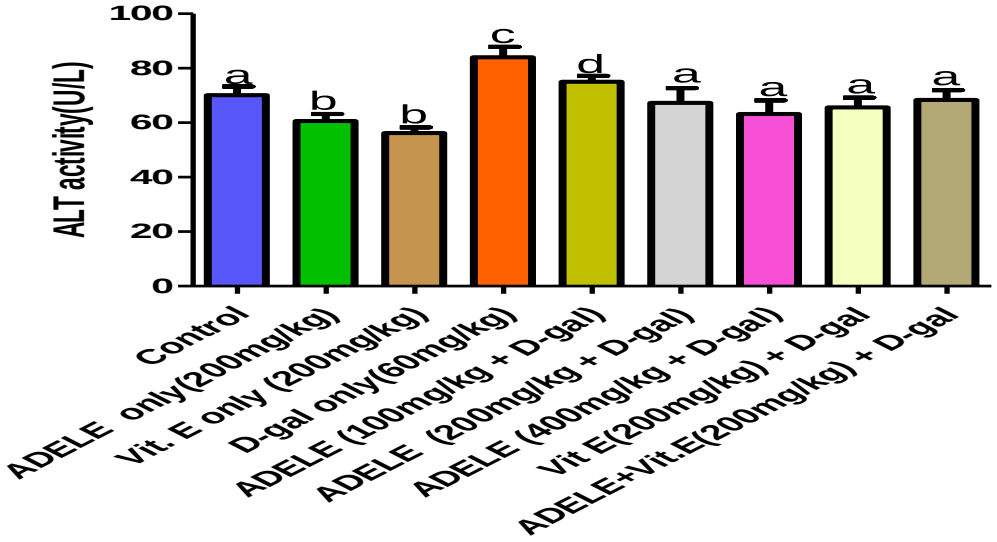

Figure 1: Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Alanine transaminase activity in serum of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

Bars with different alphabets are significantly different from each other.

Bars with the same alphabets are not significantly different from each other.

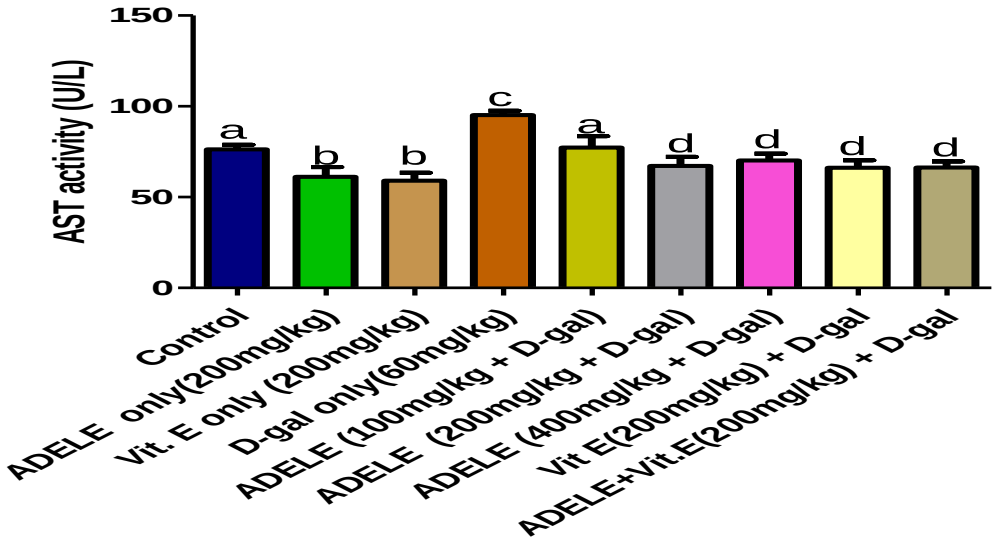

Figure 2: Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Aspartate transaminase activity in serum of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

Bars with different alphabets are significantly different from each other.

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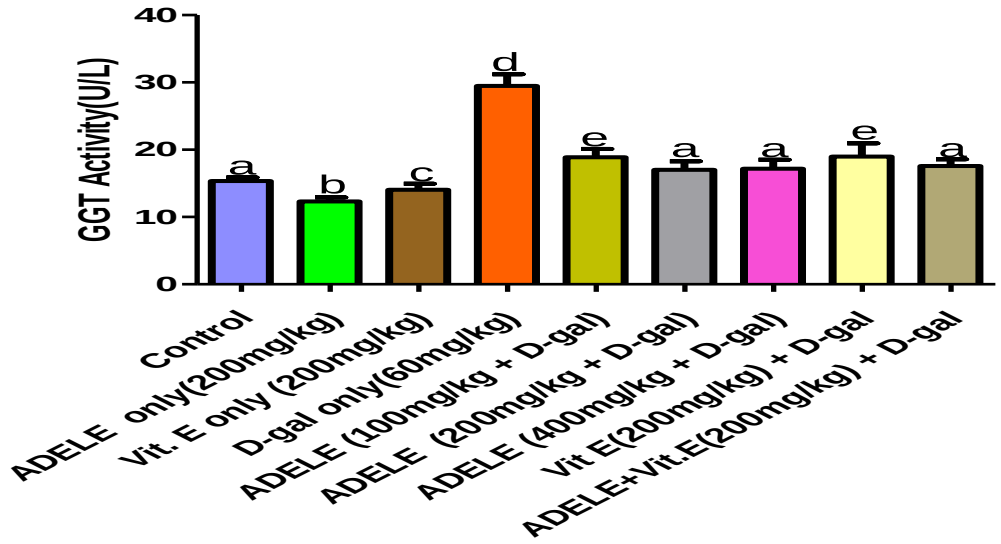

Figure 3: Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Gamma-glutamyl Transferase activity in serum of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

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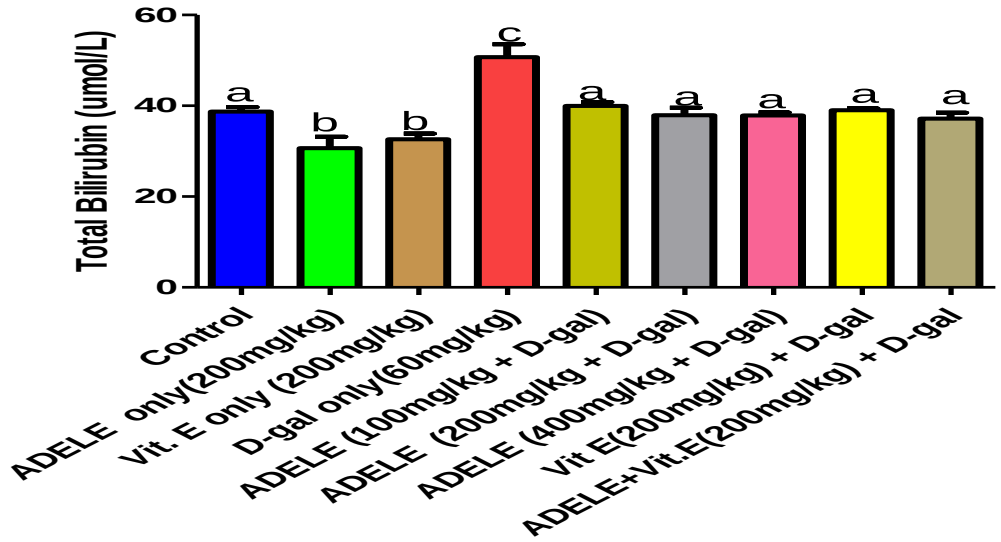

Figure 4: Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Total Bilirubin concentration in serum of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

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(B) Antioxidant Indices

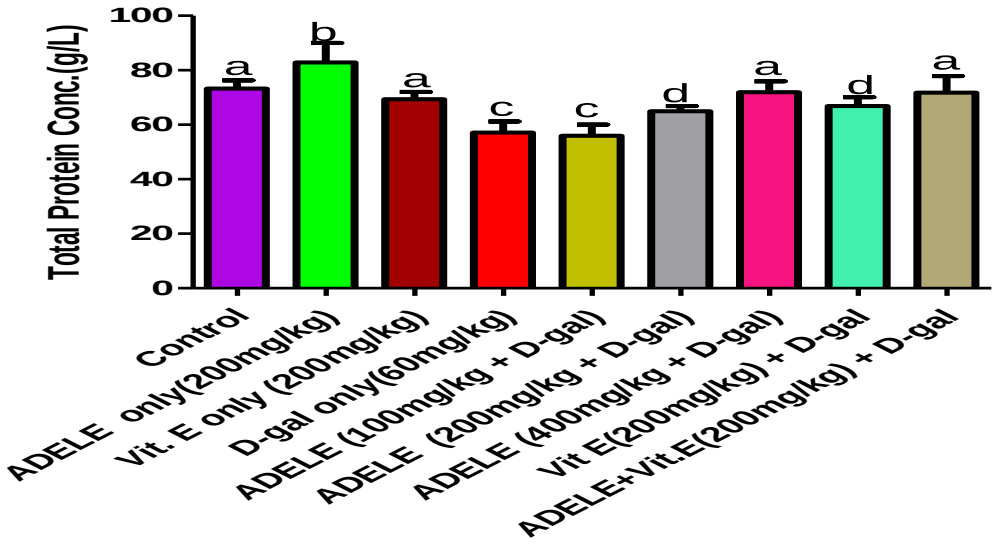

Figure 5: Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Total Protein concentration in serum of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

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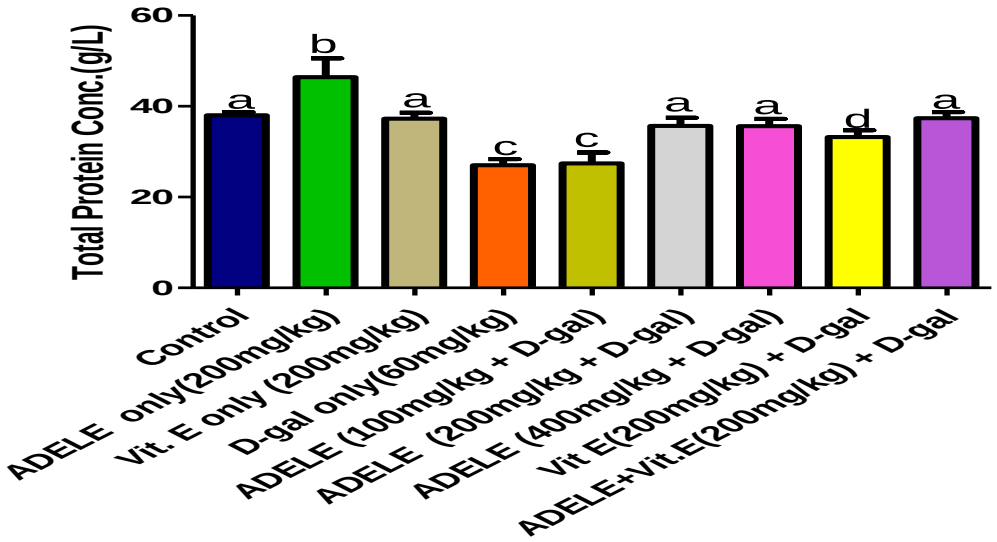

Figure 6: Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Total Protein concentration in Liver homogenates of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

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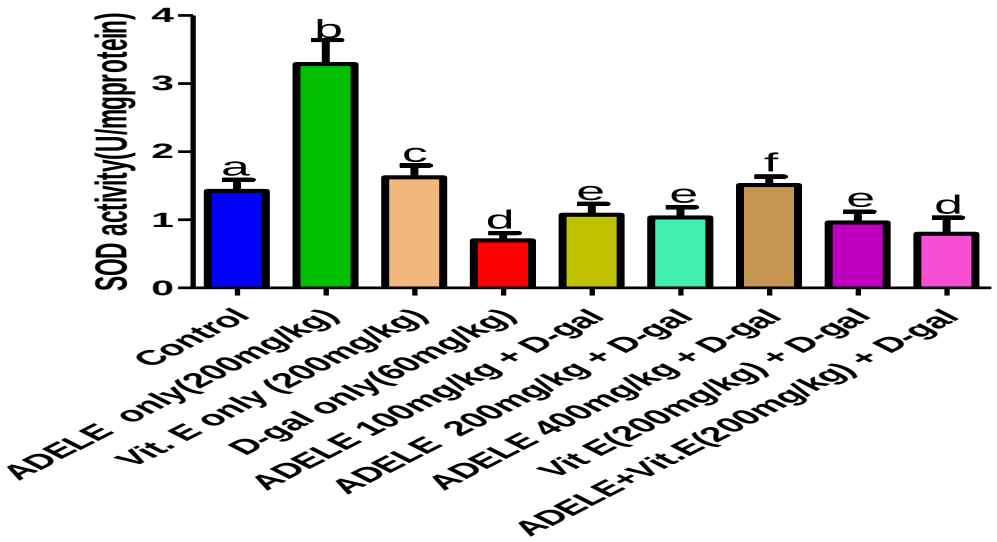

Figure 7 : Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Superoxide Dismutase (SOD) activity in Liver homogenates of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

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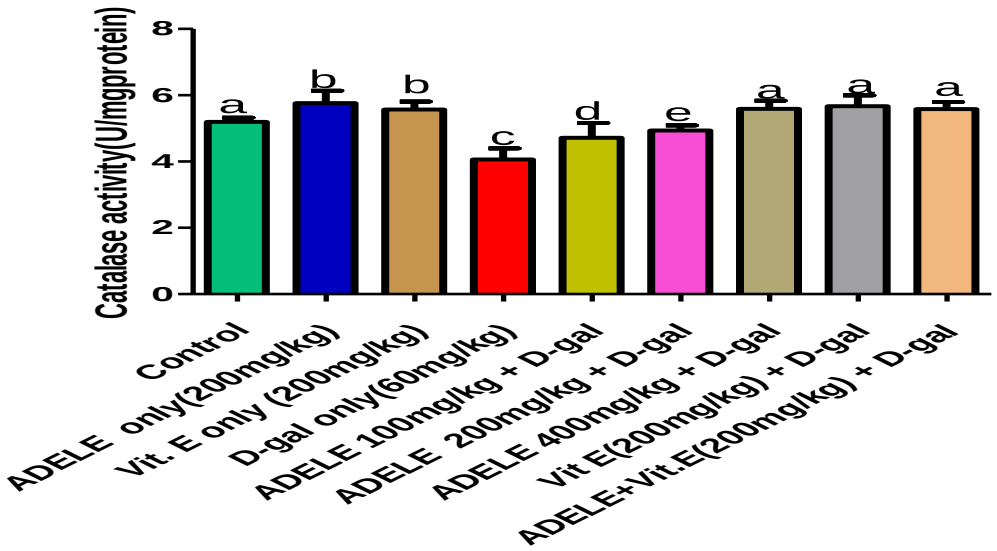

Figure 8 : Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Catalase activity in Liver homogenates of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

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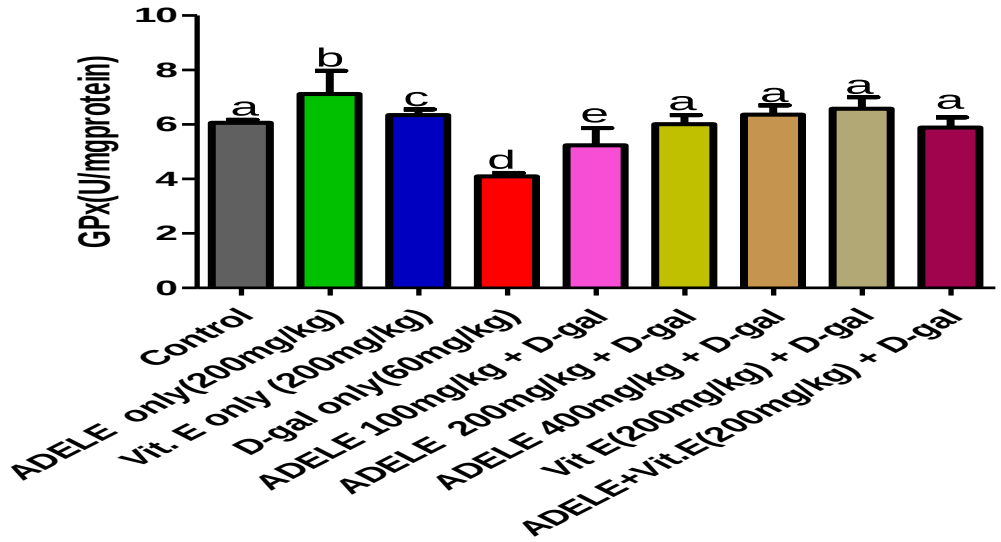


Figure 9 : Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Glutathione Peroxidase (GPx) activity in Liver homogenates of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

Bars with different alphabets are significantly different from each other.

Bars with the same alphabets are not significantly different from each other.

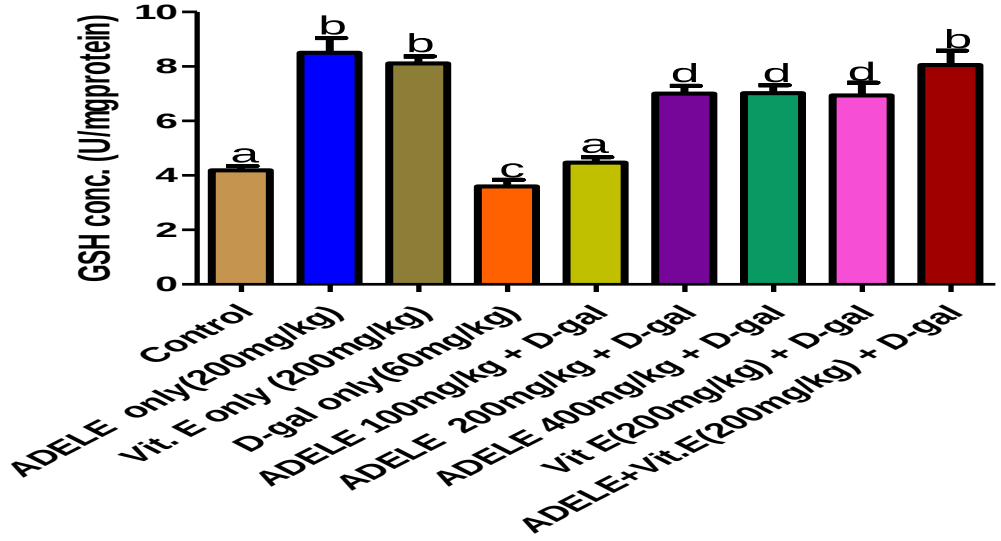


Figure 10 : Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Reduced Glutathione (GSH) concentration in Liver homogenates of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

Bars with different alphabets are significantly different from each other.

Bars with the same alphabets are not significantly different from each other.

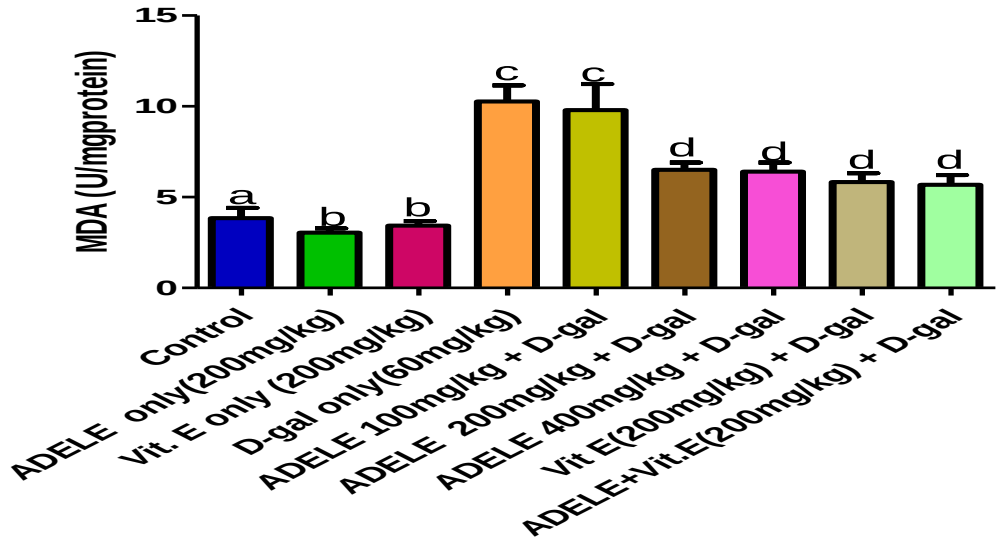


Figure 11 : Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Malondialdehyde (MDA) concentration in Liver homogenates of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

Bars with different alphabets are significantly different from each other.

Bars with the same alphabets are not significantly different from each other.

(C) Inflammatory Indices

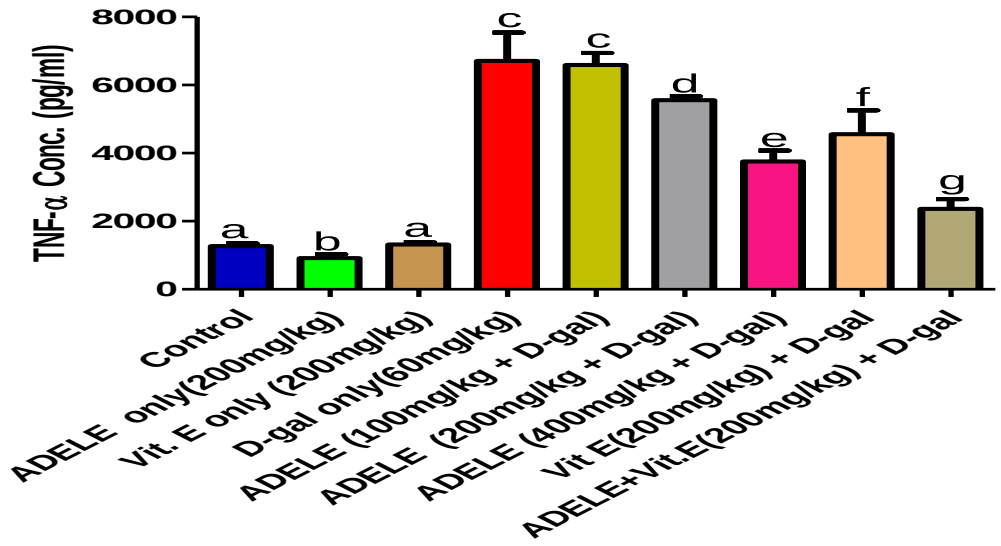


Figure 12: Effects of *Adansonia digitata* Ethylacetate Leaves Extract on Tumour Necrosis Factor (TNF-α) concentration in Liver homogenates of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

Bars with different alphabets are significantly different from each other.

Bars with the same alphabets are not significantly different from each other.

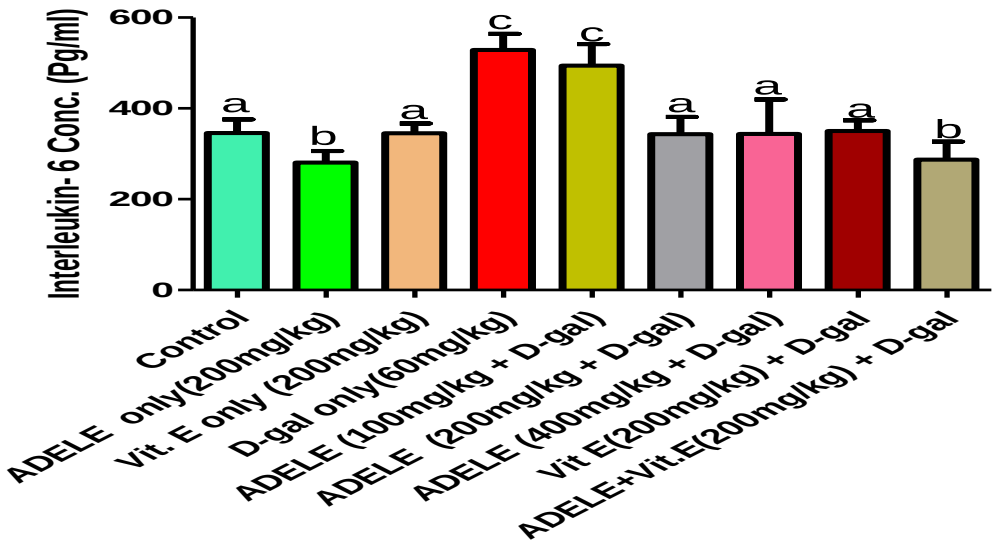


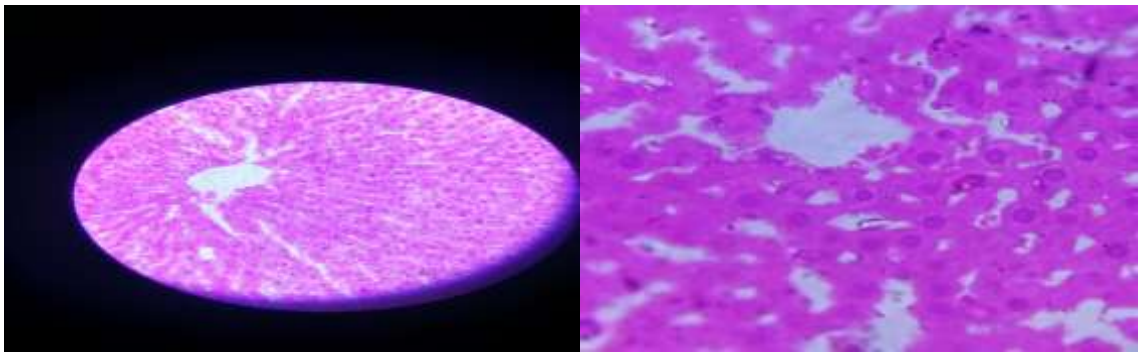
Figure 13 : Effect of *Adansonia digitata* Ethylacetate Leaves Extract on Interleukin-6 (IL-6) concentration in Liver homogenates of various treatment groups.

Level of significance was taken at (P < 0.05) for seven rats per group.

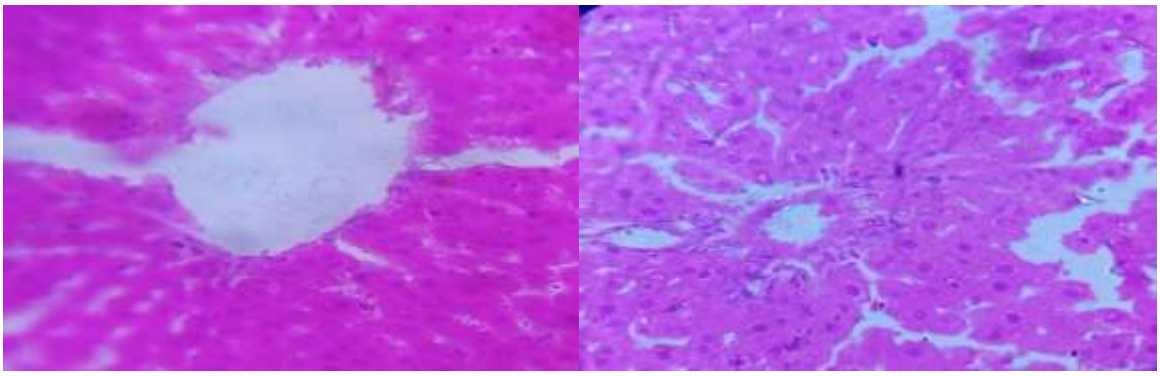
Bars with different alphabets are significantly different from each other.

Bars with the same alphabets are not significantly different from each other.

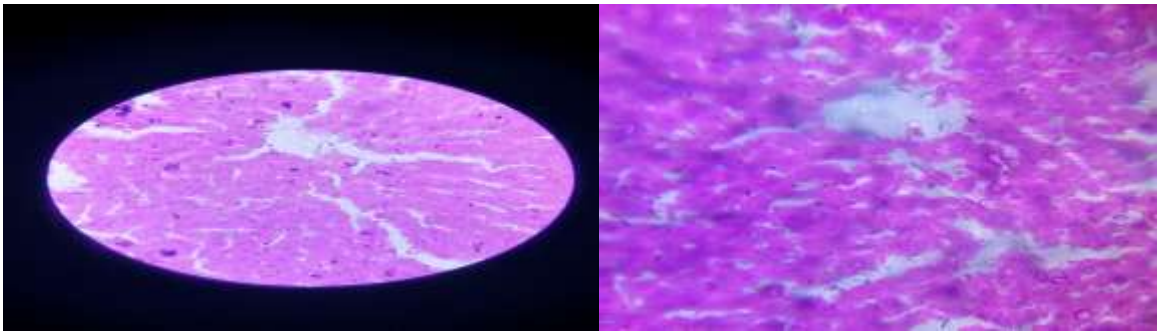
2.9. Hepatic Histopathology



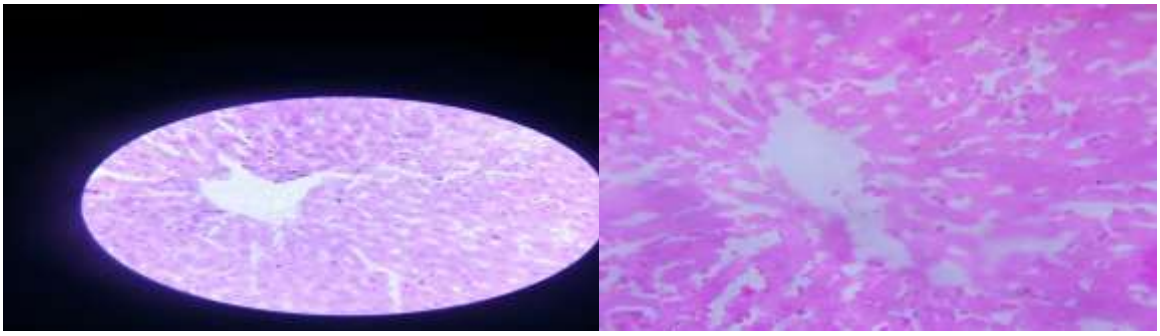
GROUP I GROUP II



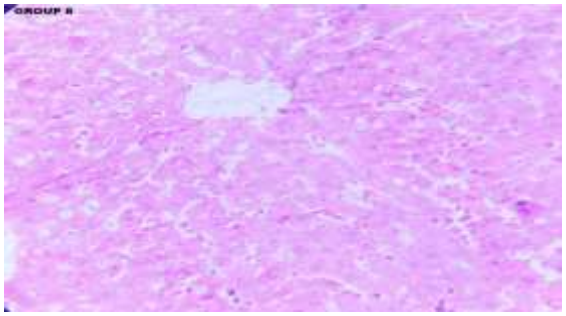
GROUP IV GROUP III



GROUP VI GROUP V



GROUP VIII GROUP VII



GROUP IX

Plate 3.4: Effects of *Adansonia digitata* Ethylacetate Leaves Extract (ADELE) on hepatic tissues of various treatment groups by haematoxylin and eosin staining (×400). Group I (Control), Group II (Extract only), and Group III (Vit. E only) showed no alteration in panoramic morphological presentation, with normal central vein and hepatocytes, which are more pronounced in Group II and Group III. Group IV (D-galactose only) showed degenerative changes in the hepatocytes characterised by abnormal enlargement of central vein and degenerated hepatocytes. Group V (ADELE 100 mg/kg + D-gal), Group VI (ADELE 200 mg/kg + D-gal), and Group VII (ADELE 400 mg/kg + D-gal) showed protection against liver degeneration and recovery of induced liver degeneration in central vein and hepatocytes, which makes the micrographs of these groups close to normal. In Group VIII (Vit. E + D-gal) and Group IX (ADELE + Vit. E + D-gal): mild degenerative changes in central vein and hepatocytes, close to normal hepatocyte architecture. Central vein (black arrow), hepatocytes (yellow arrow).

3. Discussion

Transaminases, otherwise referred to as aminotransferases, are enzymes responsible for the transfer of amino groups from particular amino acids to α-keto acids, which is essential for the metabolism of amino acids as well as the synthesis of protein. Their levels in the blood or serum correlate with

the degree of hepatic damage that has taken place, as the enzymes are released into the bloodstream (Ghanbari-Niaki et al., 2022). AST and ALT are the two clinically significant transaminases for detection of hepatic damage (Kamil et al., 2024). GGT is a liver enzyme that performs the function of transferring glutathione to other molecules, and its measurement in serum is used to detect the level of hepatic damage or damage to the bile duct. Elevated GGT level has been ascribed to hepatocyte damage (Shwe et al., 2018; Azman et al., 2021). This present study determined the effects of *Adansonia digitata* ethylacetate leaves extract (ADELE) and Vit. E on alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT) activities in serum of various treatment groups as shown in figure 1, 2, and 3 respectively. Group II (ADELE only) and Group III (Vit. E only) showed significant ($P < 0.05$) decreases in ALT, AST, and GGT activities compared with the control and other treatment groups. D-galactose occasioned a significant ($P < 0.05$) increase in these parameters in Group IV (D-galactose only) compared with the control and other treatment groups, which is an indication of hepatic damage, as these enzymes find their way into the bloodstream when there is free-radical-mediated injury to the hepatic tissue. ALT is located exclusively in the cytoplasm of hepatocytes while AST is located in both the cytoplasm and mitochondria. GGT is a sensitive biomarker for hepato-biliary diseases; its significant ($P < 0.05$) elevation in Group IV (D-galactose only) might be a result of oxidative stress or cholestasis, as oxidative stress depletes intracellular glutathione while the body responds by up-regulating GGT to break down extracellular glutathione, providing the cell with cysteine to synthesise new intracellular GSH. However, pretreated groups (Group V, VI, VII, VIII, and IX) were able to scavenge free radicals released by D-galactose and were all brought near to the control levels. The plant extract exhibited hepatoprotective property via its antiradical ability, reflecting its antioxidant and anti-ageing potential, which might be through its embedded polyphenols having down-regulated the GGT-stimulating pathway. This hepatoprotective effect of ADELE active polyphenols or their metabolites might be through up-regulation of the cytochrome P450 family of enzymes or by eliciting various hepatic biochemical pathways leading to suppression of these hepatic enzymes. The obtained result is consistent with the earlier report of Azman et al. (2021), who reported similar effects on AST and ALT activities in a D-galactose-induced animal model, and also with Musab et al. (2015), Shati et al. (2016), and Sa'id et al. (2020), who reported similar effects on ALT and AST transaminases as well as GGT activity in their various experimental rat models using *Adansonia digitata* leaves extracts.

Chronic D-galactose induction has also been attributed to elevated levels of total and direct bilirubin, which has been linked to adverse effects of oxidative stress and inflammation (Boland et al., 2014). A high level of bilirubin in the serum has been linked to liver damage, blockage of the bile duct, or excessive breakdown of red blood cells (Boland et al., 2014; Saleh et al., 2019). In this study, Group II (ADELE only) and Group III (Vit. E only) showed significant ($P < 0.05$) decreases in bilirubin concentration compared with the control and other treatment groups as shown in figure 4. However, Group IV (D-galactose only) showed a significant ($P < 0.05$) increase in bilirubin concentration compared with the control and other treatment groups, an indication of hepatotoxicity or liver damage, which is one of the hallmarks of ageing, as hyperbilirubinaemia is caused by disruption of the normal haeme metabolic pathway involving production, transportation, conjugation, biliary excretion, and hepatic uptake. Excessive haemolysis of red blood cells results in haemoglobin breakdown, which ultimately results in excessive production of bilirubin, as haeme is converted to biliverdin and finally to bilirubin. Pretreated groups (V, VI, VII, VIII, and IX) were brought near to the control levels and were not significantly ($P > 0.05$) different from the control group, reflecting the protective effects of ADELE embedded phytonutrients on the hepatocytes,

which might be through stimulation of biochemical pathways that down-regulated the production of bilirubin. A similar effect was also observed in Group II and Group III and also between Group VII and Group IX, although the effect of the extract was not concentration-dependent. The result obtained corroborated the earlier reports of Shati et al. (2016) in indole-3-acetic acid (IAA)-induced hepatotoxicity in rats treated with extract of *Adansonia digitata* leaves, as well as Musab et al. (2015) and Sa'id et al. (2020) in carbon tetrachloride (CCl_4)-induced liver damage in rats treated with different concentrations of fruit extracts of *Adansonia digitata*.

Effects of *Adansonia digitata* and Vit. E on total protein concentration in serum and hepatic tissues of various treatment groups were determined in this study as illustrated in figure 5 and 6. The ADELE-only treated group (Group II) showed a significant ($P < 0.05$) increase in total protein compared with the control and other treated groups, while Group III (Vit. E only) was not significantly different ($P > 0.05$) from the control. However, the D-galactose-only group showed a significant ($P < 0.05$) decrease in total protein compared with the control and other treated groups, but was not significantly ($P > 0.05$) different from Group V (ADELE 100 mg/kg bw and D-gal). Pretreated groups VI–IX showed elevated levels of total protein concentration compared with the D-galactose-only treated group, as the extract was able to scavenge free radicals released by D-galactose, though not in a concentration-dependent manner. A similar effect was observed between Group VII (ADELE 400 mg/kg and D-gal) and Group IX (ADELE and Vit. E and D-gal). *A. digitata* possesses secondary metabolites that might have interfered with protein metabolism resulting in up-regulation of protein synthesis, or may possess appreciable amounts of protein in its leaves, which might have enhanced protein concentration in the rats. Makena et al. (2021) also reported similar effects in lead-induced hepato-renal damage in experimental rats treated with *Adansonia digitata* fruit pulp extract.

Reactive oxygen species (ROS) have been implicated in the aetiology of ageing, and the antioxidant system has been reported to control ageing effects occasioned by ROS by decreasing the rate of free-radical-mediated oxidative damage, hence increasing the life expectancy of an organism (Kazeem et al., 2012). Ageing is a complex and pleiotropic phenomenon, a natural and irreversible process that affects living organisms by adversely impacting tissue and cell functionality and morphology and is responsible for various disease conditions like hypertension, atherosclerosis, dementia, diabetes, osteoporosis, and cancer, with ever-increasing economic impact on the health of people worldwide (Tartiere et al., 2024). The effect of ethylacetate leaf extract of *A. digitata* on superoxide dismutase activity in the liver of D-galactose-treated Wistar rats was ascertained in this study as shown in figure 7. Group II (ADELE only) and Group III (Vit. E only) showed significant ($P < 0.05$) increases in SOD activity compared with the control and other treatment groups, an indication of build-up of the innate antioxidant system, while Group IV (D-galactose only) showed a significant ($P < 0.05$) decrease in SOD activity in the liver compared with the control and other treatment groups, which is an indication of free-radical-mediated oxidative stress as excessive free radicals have overwhelmed the innate antioxidant system. Reduction in SOD activity can also be caused by direct oxidative damage to this enzyme, its utilisation or consumption during the neutralisation process, or via down-regulation of its gene expression. However, pretreated groups (V, VI, VII, VIII, and IX) showed significant ($P < 0.05$) elevation in SOD activities compared with Group IV (D-galactose only) and were restored near to the control level. Secondary metabolites in ADELE, especially flavonoids, might have elicited various biochemical pathways leading to up-regulation of SOD activity in Group II and the pretreated groups. The result obtained in this study is consistent with the earlier reports of Atuadu et al. (2020), who reported elevated SOD activity in lead-induced neurotoxicity in an experimental animal model treated with *Adansonia digitata*

aqueous leaves extract, and Uhuo et al. (2022) in a doxorubicin-induced cardiotoxicity rat model treated with ethanol leaves extract of *Adansonia digitata*.

Catalase is a key enzyme present in almost all living cells, protecting cells from damage by catalysing the breakdown of hydrogen peroxide to give oxygen and water. It is an important part of antioxidant systems in the cell, having high catalytic rates and capable of converting over one million hydrogen peroxide molecules per second (Arya et al., 2018; Baker et al., 2023; Rasheed, 2024). Reduction in the level of catalase has been reported in various oxidative stress-related diseases, while chronic D-galactose induction has also been reported to decrease catalase activity (Hadzi-Petrushev et al., 2015; Chattipakorn et al., 2018). As illustrated in figure 8, Group II (ADELE only) and Group III (Vit. E only), showed a significant ($P < 0.05$) increase in catalase activities compared with control and other treatment groups, while D-galactose occasioned a significant ($P < 0.05$) decrease in catalase activity in Group IV (D-galactose only) when compared with the control and other treatment groups, an indication of overwhelming of the innate antioxidant system by free radicals. Moreover, excessive free radicals released might have directly interacted with and inhibited catalase enzyme, or extreme hydrogen peroxide (H_2O_2) overload from D-galactose might have caused 'suicide inactivation' of catalase enzyme (Garcia-Molina et al., 2005). However, pretreated groups (V, VI, VII, VIII, and IX) were able to resist the free-radical oxidative assault by D-galactose and were not significantly ($P > 0.05$) different from the control group. This reflects the antioxidant potential and possible anti-ageing effects of ADELE secondary metabolites, as various polyphenols embedded in the plant extract might have elicited catalase up-regulation, leading to the obtained antioxidant effect. Polyphenols have been reported to be responsible for various therapeutic and medicinal effects associated with medicinal plants, making them potent alternative choices for the treatment and management of oxidative stress and various degenerative diseases (Adedosu et al., 2017). This result also corroborates the earlier reports of Adegoke et al. (2017) and Uhuo et al. (2022), who reported similar effects in doxorubicin- and sodium arsenite-exposed experimental rats treated with *Adansonia digitata* leaves extract.

Glutathione peroxidase (GPx) is a tripeptide of three amino acid residues (glutamate, cysteine, and glycine). It reduces hydrogen peroxide (H_2O_2) to molecular oxygen and water using glutathione as an electron donor. D-galactose administration has been linked to elevated levels of oxidative stress and age-related damage (Cui et al., 2006; Hadzi-Petrushev et al., 2015; Mumtaz et al., 2023). As illustrated in figure 9, Group II (ADELE only) showed significant ($P < 0.05$) elevation in GPx activity compared with the control and other treatment groups, while Group III (Vit. E only) was not significantly ($P > 0.05$) different from the control. D-galactose occasioned a significant ($P < 0.05$) decrease in GPx activity in Group IV (D-galactose only) when compared with the control and other treatment groups, indicating free-radical-mediated oxidative stress overwhelming the innate antioxidant system. GPx is an enzyme that depends on selenium to reduce peroxides; excessive free radicals released by D-galactose in this study, especially H_2O_2 and lipid peroxides, might have directly oxidised and deactivated GPx. However, pretreated groups (V, VI, VII, VIII, and IX) were able to scavenge free radicals released by D-galactose and were not significantly ($P > 0.05$) different from the control. ADELE active secondary metabolites boosted GPx activity, resulting in its up-regulated activity, or activated biochemical pathways that led to reactivation of GPx. This corroborates ADELE's folkloric use as an anti-ageing remedy and its possible utility in the treatment and management of other oxidative stress-related diseases. This present study is consistent with earlier reports of Otong et al. (2021), Dare et al. (2021), and Uhuo et al. (2022), who reported similar effects on GPx activities and other antioxidant enzymes in experimental rats treated with different solvent extracts of *Adansonia digitata* leaves.

Reduced glutathione (GSH) is a non-enzymatic antioxidant molecule present in mammalian cells. GSH protects cells by acting directly as an antioxidant against free radicals and pro-oxidants. It can also act as a cofactor for enzymes responsible for detoxification, such as glutathione peroxidase, glyoxalase, and glutathione-S-transferase (Averill-Bates, 2023). Oxidative stress has been reported to reduce the level of GSH by impairing its regeneration after its consumption as an antioxidant (Waggiallah & Alzohairy, 2011). D-galactose exposure in experimental animals has been reported to reduce the level of GSH by elevating oxidative stress (Cui et al., 2006; Shi et al., 2024). As shown in figure 10, a significant ($P < 0.05$) increase in GSH concentration was observed in Group II (ADELE only) and Group III (Vit. E only) when compared with the control and other treatment groups. However, Group IV (D-galactose only) showed a significant ($P < 0.05$) decrease in GSH concentration compared with the control and other treatment groups, indicating that, free-radical-mediated oxidative stress had consumed GSH and drastically reduced its hepatic concentration. Moreover, free radicals might have caused GSH to be oxidised to glutathione disulfide (GSSG) via glutathione peroxidase activity for neutralisation of excess lipid hydroperoxides or hydrogen peroxide (H_2O_2), or might have directly attacked the GSH pool. Pretreated groups (V, VI, VII, VIII, and IX) up-regulated the reduced GSH concentration near to the control levels. The GSH restoration effect of ADELE was dose-dependent in liver tissues, while Groups VIII and IX were not significantly ($P > 0.05$) different from the control group. ADELE showed properties that up-regulated GSH concentration or stimulated processes resulting in elevated GSH pool, leading to concentration-dependent antiradical effects in this study, and also corroborating its local use as an anti-ageing remedy. The result obtained in this study corroborates the earlier report of Dare et al. (2021), who reported similar effects on GSH concentration in rat models treated with aqueous extract of *Adansonia digitata* leaves, and also El-Shora et al. (2021), who reported the beneficial effect of the methanolic leaf extract of *Allium hookeri* on stimulating glutathione biosynthesis and preventing impaired glucose metabolism in type 2 diabetes.

Malondialdehyde (MDA) is a measurable end product of lipid peroxidation and a key biomarker for oxidative stress in biological systems. MDA is a toxic compound with the potential to damage proteins and DNA (Cordiano et al., 2023). Elevated levels of MDA have been reported in chronic D-galactose-induced animal models (Ho et al., 2003; Okechukwu & Ijeh, 2024). As shown in figure 11, Group II (ADELE only) and Group III (Vit. E only) showed significantly ($P < 0.05$) decreased MDA concentrations compared with the control and other treatment groups. D-galactose administration occasioned a significant ($P < 0.05$) elevation in MDA concentration in Group IV (D-galactose only) compared with the control and all other treatment groups, which is an indication of lipid peroxidation in hepatic cell membrane lipid bilayers and other structural and functional lipids in the hepatic tissue. Moreover, ROS might have broken down polyunsaturated fatty acids (PUFAs), particularly arachidonic acids, in membranes of the hepatic cells. However, pretreated groups (V, VI, VII, VIII, and IX) were resistant to D-galactose-induced oxidative stress effects on lipids, as a result of possible build-up of the antioxidant system by ADELE secondary metabolites preventing peroxidation of structural, membrane, and other lipid components in the liver cells, or down-regulation of the process that stimulated excessive breakdown of PUFA, hence suppressing lipid peroxidation. Peroxidation of lipids has been reported to be one of the hallmarks of ageing, as natural agents with good antioxidant properties have also been reported to serve as anti-ageing remedies (Ho et al., 2003). The result obtained in this present study is consistent with earlier reports of Atuadu et al. (2020) and Akintola et al. (2021), who reported decreases in MDA concentration in *Adansonia digitata* leaves extracts in their various experimental rat models.

Tumour Necrosis Factor (TNF-α) is a pleiotropic pro-inflammatory cytokine produced by the immune system. It normally induces inflammation when it binds to its receptor on other cells and is also essential for fighting infections. An increase in TNF-α levels has been implicated in inflammatory conditions, various autoimmune conditions, and in ageing (Baradaran et al., 2022; Bazile et al., 2024). D-galactose has been reported to elevate the level of TNF-α in different ageing animal models via activation of inflammatory pathways and promotion of oxidative stress (Lee et al., 2017; Kumar et al., 2022). In this study, as shown in figure 12, a significant ($P < 0.05$) decrease in TNF-α concentration was observed in Group II (Extract only) and Group III (Vit. E only) compared with the control and other treatment groups. However, Group IV (D-galactose only) showed a significant ($P < 0.05$) increase in TNF-α concentration compared with the control and other treatment groups, an indication of activation of inflammatory pathways resulting in elevated TNF-α, an inflammatory cytokine whose elevated levels have been implicated in ageing. TNF-α might have induced production of ROS via NADPH oxidase, acting as a positive feedback mechanism to sustain NF-κB inflammatory pathways in the hepatic tissue. However, pretreated groups (V, VI, VII, VIII, and IX) showed a concentration-dependent decrease in TNF-α concentration. ADELE embedded phytonutrients might have interfered with the inflammatory response milieu (NF-κB or JNK), resulting in down-regulation of TNF-α concentration and other inflammatory-associated factors. The obtained result for TNF-α in this study is consistent with the earlier report of Lee et al. (2017), who reported a significant ($P < 0.05$) decrease in TNF-α concentration in a D-galactose-induced experimental rat model treated with extract of medicinal plants, as well as Kumar et al. (2022), who reported significant ($P < 0.05$) increases in TNF-α concentration in D-galactose-induced rat models, and also corroborates the earlier report of Ebaid et al. (2019), who reported decreased levels of TNF-α in streptozotocin-induced diabetic rats treated with methanolic extract of *Adansonia digitata* leaves.

Interleukins (ILs) are proteins and cytokines acting as cell-to-cell messengers inside the immune system in order to regulate cellular responses such as growth and cell differentiation (Vaillant & Qurie, 2022). Interleukin-6 (IL-6) acts as a pro-inflammatory molecule produced by immune and non-immune cells, triggering various biological effects such as inflammation. Elevated levels of interleukin-6 have been reported in various inflammatory conditions such as sepsis, cardiovascular diseases, and ageing (Rincon, 2012). IL-6 is reported to be crucial for liver cell homeostasis and is also a potent hepatic cell mitogen (Schmidt-Arras & Rose-John, 2016). Administration of D-galactose has been reported to increase interleukin-6 levels, promoting oxidative stress, inflammation, and ageing (Gama et al., 2025). As illustrated in figure 13, Group II (ADELE only) significantly ($P < 0.05$) decreased IL-6 concentration in hepatic tissues compared with the control and other treatment groups. D-galactose occasioned a significant ($P < 0.05$) increase in IL-6 concentration in Group IV (D-galactose only) compared with the control and other treatment groups, indicating that severe inflammation had taken place in the hepatic cells of D-galactose-exposed rats. Free radicals might have activated the pro-inflammatory NF-κB pathway in the hepatic tissues, resulting in elevated IL-6. Pretreated groups (VI, VII, VIII, and IX) showed decreased concentrations of IL-6 cytokines. Although the effect of ADELE was not concentration-dependent, ADELE active compounds or their metabolites displayed good anti-inflammatory potential by down-regulating IL-6 cytokine production, or might have interrupted the biochemical pathway (NF-κB) responsible for excessive production of IL-6 cytokines, hence protecting hepatocytes from free-radical-mediated oxidative damage. This result is consistent with the earlier report of Ebaid et al. (2019), who reported down-regulation of IL-6 cytokine in streptozotocin-induced diabetic rats treated with various doses of methanolic extract of *Adansonia digitata* leaves extract.

Interestingly, histological examination of hepatic tissues in this present study corroborates the potential of ADELE as a possible anti-ageing remedy. Group I (Control), Group II (Extract only), and Group III (Vit. E only) showed no alteration in panoramic morphological presentation, with normal central vein and hepatocytes, which are more pronounced in Group II and Group III, indicating that active secondary metabolites of ADELE have improved the structural architecture of the hepatocytes. ADELE improved the cellular and structural integrity of hepatocytes compared with the control group, a mechanism which might be employed for its anti-ageing potential. However, in Group IV (D-galactose only), degenerative changes in the hepatocytes characterised by abnormal enlargement of central vein and degenerated hepatocytes were observed as a result of the effects of free radicals occasioned by D-galactose, disrupting the architecture of the hepatic cells. Furthermore, Group V (ADELE 100 mg/kg bw), Group VI (ADELE 200 mg/kg bw), and Group VII (ADELE 400 mg/kg bw) showed protection against liver degeneration and recovery of induced liver degeneration on central vein and hepatocytes, making the micrographs of these groups close to normal, while Group VIII (Vit. E and D-gal) and Group IX (ADELE 100 mg/kg bw and Vit. E 100 mg/kg bw and D-gal) showed mild degenerative changes in central vein and hepatocytes, reflecting the ability of the phytonutrients of ADELE to protect the hepatocytes from D-galactose-induced oxidative damage, marking them almost normal in appearance. The result obtained in this present study is consistent with the earlier report of Liu et al. (2025), who reported dose-dependent restoration of distorted hepatic tissue treated with plantagoside in D-galactose-induced ageing, and also with Han et al. (2025), who reported protective effects of *Gastrodia elata* extract in D-galactose-induced liver injury in mice based on the PI3K/Akt signalling pathway, as well as Wei et al. (2025), who reported delayed effects of anthocyanins in D-galactose-induced mouse liver ageing by regulating the NF-κB/IKK signalling pathway, and Hanafy et al. (2016), who reported hepatoprotective effects of *Adansonia digitata* in acetaminophen-induced hepato-histological toxicity.

4. Conclusion

The results obtained in this study showed D-galactose's ability to induce redox imbalance, alter the antioxidant system, occasion oxidative stress, and cause tissue toxicity, while ethylacetate leaf extract of *Adansonia digitata* linn exhibited antioxidant, anti-inflammatory, and hepatoprotective effects. Hence, its bioactive components may be explored for the treatment and management of oxidative stress-related and inflammatory disorders, and may also serve as a promising lead for drug discovery towards ageing and its associated disorders. However, this study may also be carried out on female Wistar rats to explore the possible differences regarding the sex of the experimental rats.

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