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ANTI-AGING EFFECTS OF METHANOLIC LEAVES EXTRACT OF VERNONIA AMYGDALINA ON D-GALACTOSE INDUCED AGING IN MALE WISTAR RATS

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ABSTRACT

Vernonia amygdalina has shown promising anti-aging effects in folk medicine with little or no scientific basis. This study investigated anti-aging effects of Methanol Leaves Extract of *Vernonia amygdalina* (MLEVA) on D-galactose induced aging in male Wistar rats. Thirty-two rats weighing averagely 200g were randomly selected into four groups of eight rats each. Group I(Control)-received feed and distilled water only, Group II–received 2ml of D-galactoseonly at 300mg/kg body weight (bw) daily, Group III and IV-received, D-galactoseactose, followed by MLEVA at 200 and 300mg/kg bw respectively, for six weeks. Biochemical parameters were determined in serum, liver and kidney homogenates using spectrophotometric techniques. Superoxide dismutase (SOD) and Catalase (CAT) activities, as well as Reduced glutathione (GSH) Concentration, were significantly ($P<0.05$) decreased in group II compared with control, but were significantly restored ($P<0.05$), in groups III and IV compared with group II. Furthermore, Creatinine and Urea concentrations were significantly ($P<0.05$) elevated in group II compared with control, but were significantly restored ($P<0.05$), in a dose dependent approach in groups III and IV, compared with group II. Moreover, Alanine transaminase (ALT) and Aspartate transaminase (AST) activities were significantly ($P<0.05$) increased in group II compared with control, but were restored significantly ($P<0.05$) in a dose dependent approach, in groups III and IV, compared with group II. Malondialdehyde (MDA) concentration was significantly ($P<0.05$) elevated in group II compared with control, but groups III along with group IV showed a significant ($P<0.05$) reversal compared with group II. *Vernonia amygdalina* exhibited anti-aging effects via attenuation of D-galactoseactose-mediated oxidative stress, validating its folkloric use as an anti-aging plant, and its possible potential in amelioration and treatment of other oxidative stress and age-related diseases.

Keywords: *Vernonia amygdalina*, D-galactoseactose, free radical, oxidative stress, anti-aging.

INTRODUCTION

The *Vernonia amygdalina* is a member of the daisy family (Compositae or Asteraceae) and a widely grown shrub plant in Africa consumed as vegetable and has high medicinal value. The leaves are useful components for herbal and traditional medicine constitution (Ifeoluwa *et al*., 2018).

Aging is a multifaceted process in which progressive loss of physiological integrity, susceptibility to sickness, and lower quality of life are connected with or accountable for it. Brain ageing is the cornerstone of aging, as seen by mental deficiencies such as cognitive decline. Evidence indicate that aging-related cognitive deficits are linked to biological changes such as increased oxidative stress and apoptosis (Pourmemar *et al*., 2017). In other words, aging is a multidimensional, time-dependent process that is caused by a progressive loss of physiological integrity, resulting in functional impairment and reduced quality of life. One of the cardinal aspects of aging is brain ageing, which manifests itself in a wide variety of behavioral abnormalities such as anxiety and impaired cognitive performance. Improvements in brain structural integration, reductions in neurogenesis, lipid peroxidation, oxidative stress, mitochondrial dysfunction, decreases in neurotransmitter levels, and beta amyloid (A) overproduction have all been proposed as significant mediators of brain aging and age-related neurological conditions (Sadigh-Eteghad *et al*., 2017).

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According to Bo-Htay *et al*. (2018), aging is a significant independent impairment, morbidity, and mortality risk factor. Post-mitotic cells, particularly those in the heart, have a special risk for age-related degeneration. As the prevalence of heart disease rises fast with the aging population, more research on the causes of age-related cardiac susceptibility and prospective therapeutic approaches is needed to avert rising levels of heart disease. Heart failure, myocardial infarction, stroke, diabetes mellitus, cancer, Alzheimer's, Parkinson's disease, osteoporosis, and osteoarthritis are common causes of death among the elderly. Many strategies for extending human life have lately been proposed, with varied degrees of effectiveness (Kolovou *et al*., 2014).

Mitochondrial dysfunction, as well as other types of age-related neurodegeneration, are essential factors in brain aging and senescence. Increased brain oxidative stress is caused by a vicious cycle of ROS-induced mitochondrial ROS release, which leads to brain aging or senescence. Finally, as the brain ages, it experiences cognitive decline, which is the primary sign of most neurodegenerative illnesses in the elderly. The key underlying processes of brain aging are connected to increased oxidative stress and mitochondrial dysfunction. Both H2O2 and OH- are reactive oxygen species (ROS) that may induce lipid peroxidation in cell membranes as well as disturb redox equilibrium, causing neuronal damage (Shwe *et al*., 2018).

D-galactose has been demonstrated to cause aging-like effects in experimental animals. In reality, since the early 1990s, D-galactose for animal aging models has been widely employed in antiaging research across the globe. A D-galactose animal model has been used in several research to examine the aging processes of the brain and heart. D-galactose has also been utilized to represent liver and renal aging in other investigations (Aydin *et al*., 2012; Saleh *et al*., 2019; Umbayev *et al*., 2020). D-galactose treatment has been shown to promote oxidative stress expression while decreasing antioxidant expression (Bo-Htay *et al*., 2018). D-galactose has been used in animal models to explore the mechanisms of brain ageing and anti-aging therapy, according to their research (Pourmemar *et al*., 2017). D-galactose mimics many behavioral and molecular aspects of brain ageing in rodent models, according to their research (Pourmemar *et al*., 2017). From a behavioral standpoint, D-galactose causes spatial and recognition memory impairment in rats. On the other hand, mounting evidence indicate that persistent systemic D-galactose infusion increases oxidative stress and its consequences. Galacticol, which accumulates in the cell and produces reactive oxygen spices, is likewise formed by high d-galactose concentrations (ROS). It also increases malondialdehyde (MDA) levels and total antioxidant capacity (TAC), while decreasing the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-px), monoamine oxidase (MAO) B, and catalase enzymes, all of which contribute to oxidative stress. D-galactose also promotes apoptosis, an essential factor in brain aging, by activating the NF-kB pathway and increasing the protein production of Bax and caspase-3. Increased oxidative damage and apoptosis may both play a role in the development of early ageing cognitive impairment, according to evidence. The Management, prevention and treatment of diseases related to oxidative stress has been carried out using orthodox drugs with much side effects hence, the need to source for natural products of plant origin which are believed to have little or no side effect (Ayoka *et al.,* 2022). The *Vernonia amygdalina* is a medicinal plant which is used in traditional medicine to treat different infections. The aqueous and ethanolic extracts of this plant were phytochemically studied and tested against various microorganisms responsible for different human infections (Arekemase *et al*., 2020). Therefore the aim of the study is to determine the effect of *Vernonia amygdalina* on oxidative damage produced by D-galactose-induced ageing and its effect on the kidney and liver of male albino rats.

MATERIALS AND METHODS

Chemicals and Reagents

The reagents and chemicals used for this research work were all of analytical grades. They were obtained from Sigma Aldrich Company Louis, USA and also from Randox Laboratory Limited Ardmore Diamond Road, Crumlin, United Kingdom.

Experimental Animals

Healthy male Albino rats weighing between (180-200g) that has not been subjected to previous experimental procedures were obtained from the Animal house, Physiology Department, Ladoke Akintola University of Technology. Their weights were recorded before the experiment began, and they were kept in plastic cages. They were acclimatized to Laboratory condition of 12hours light and 12hours darkness for two weeks. All the procedures that were involved in the use of laboratory animals were followed according to the Institute Animal Ethics Committee regulation.

Approval of ethics

The Animal Care and Ethics Committee at Ladoke Akintola University of Technology authorized the use of all animals.

Designing of Experiments

Thirty-two male albino rats were randomly divided to four groups of eight rats each and given the following treatments for six weeks:

Group I (Control) - received standard rat pellet and distilled water only.

Group II (D-galactoseonly) –received daily dose of D-galactoseat 2ml of 300mg/kg bw dissolved in distil water for aging induction.
Group III (D-galactose + 200mg/kg bw *V. amygdalina*)– received daily dose of D-galactose at 300mg/kg bw, followed by oral administration of 200mg/kg bw of *Vernonia amygdalina.*
Group IV (D-galactose + 300mg/kg bw *V. amygdalina*)- received daily dose of D-galactose at 300mg/kg bw, followed by oral administration of 300mg/kg bw of *Vernonia amygdalina.*

Aging induction using D-galactose
Aging was induced in the rats by dissolving D-galactose(300mg/kg bw) in distil water and daily dose of 2ml were being administered orally to the rats for the period of six weeks (Parameshwaran *et al.,* 2010; Budni *et al.,* 2016.)

Plant sample collection and preparation
Fresh and matured leaves of *Vernonia amygdalina* were collected from the farmland of Ladoke Akintola University of Technology (LAUTECH) in Ogbomoso and recognized by a taxonomist at the botany division of the same institution's Pure and Applied Biology Department. They were air-dried in the shade for four weeks at room temperature., then crushed into powdered form using an electric blender to increase the surface area available for solvent extraction. 1 kilogram of crushed leaves was steeped in 5 liters of methanol for 10 days, then filtered using Whatmann filter paper number 1 and concentrated in a rotary evaporator at 30°C, then allowed to evaporate at room temperature to yield crude methanolic extract.

Collection and preparation of Tissues
The animals were fasted overnight, weighed before being sacrifice via cervical dislocation. The hepatic and renal tissues of the rats were quickly removed and rinsed repeatedly in 1.15% KCl, placed in sterilized sample bottles. They were homogenized with 0.1M phosphate buffer (pH 7.0) using electric homogenizer.

Collection and Preparation of blood Serum
The blood samples of the rats were collected using 5ml sterilized needle and Syringe via cardiac puncture, the blood was drained into sterilized plain bottles and allowed to stand for 30 minutes. The samples were centrifuged at 3000 rpm for the period of 10 minutes. The serum was picked using micropipette to another sterilized sample bottles, stored in refrigerator for determination of biochemical parameters.

Determination of Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinine concentration
The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined in the plasma according to the methods of Reitman and Frankel as described by Ochei and Kolhatkar (2008). Urea concentration was determined by Urease-Berthelot method. Creatinine concentration was determined by modified method of Jaffe (1886) and Bartels (1972).

Estimation of SOD, MDA, GSH and Catalase
The activity of superoxide dismutase (SOD) was determined by using the method of Misra and Fridovich (1972). Lipid peroxidation was determined based on the principle of Varshney and Kale (1990). Estimation of lipid peroxidation was based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) forming a MDA-TBAR adduct that absorbed strongly a 532nm. Reduced glutathione (GSH) level in the liver was assayed following the method of Ellman (1959), modified by Hissin and Hilf (1976). Catalase activity was determined by the method of Aebi (1983).

Histological Study
At the end of the experiments, hepatic and renal Specimens were collected from rats of various treated groups, fixed in 10% buffered formosaline at pH 7.0, it was dehydrated in ethyl alcohol, cleared in Xylol and embedded in paraffin 4-6 microns thickness was prepared and stained using heamatoxylin and eosin for the examination of both hepatic and renal tissues (Bancroft and Gamble, 2008).

STATISTICAL ANALYSIS

The data was given in the form of Mean Standard Error of Mean (SEM). The statistical analysis was performed using Graphad prism 5 software. To compare relative expression levels for various groups, one-way analysis of variance (ANOVA) was utilized, followed by a post hoc test (Turkey).

RESULTS AND DISCUSSION

The present study findings presented in Table 1, Figure 1 and Table 3, both kidney and liver MDA concentrations were significantly elevated in group II (D-galactose only), as a result of free radical damages to lipids, occasioned by D-galactose when compared with other groups, but were reversed on treatment with 200mg/kg and 300mg/kg bw extracts respectively, when compared with group II (D-galactose only). This demonstrated potential inhibitory effects of *Vernonia amygdalina* on lipid peroxidation chain reaction and suggest its anti peroxidative effects. Flavonoids present in methanol leave extract or its metabolites might have inhibited the process of lipid peroxidation, because they have been reported to have reasonable significant antioxidant activities (Igile *et al.,* 1994).

The results (Table 1, Fig. 5 and Table 2) for both renal and hepatic tissues, Superoxide dismutase (SOD) activities of

the animals in Group II (D-galactose only) considerably decreased compared to those in Group I (Control), the treated groups (Group III & IV) with 200mg/kg body weight and 300mg/kg body weight of *Vernonia amygdalina* extract respectively showed significant increase in the superoxide dismutase activities after treatment when compared with rats in D-galactose only treated group at P<0.05. Furthermore, in fig. 6 and 11, Catalase activities in both renal and hepatic tissues of the animals in Group II (D-galactose only) considerably decreased compared to those in Group I (control), the treated groups (Group III & IV) with 200mg/kg bw and 300mg/kg bw of *Vernonia amygdalina* extract respectively showed significant increase in catalase activities after treatment when compared with rats in D-galactose only treated groups at P>0.05. The obtained result for both Catalase and Superoxide dismutase implies possible antioxidant and free radical scavenging potential of the *Vernonia amygdalina* extract. Also, the plant extract might have elicited a biochemical pathway that increased superoxide dismutase and Catalase activities hence, boost antioxidant status of the rats.

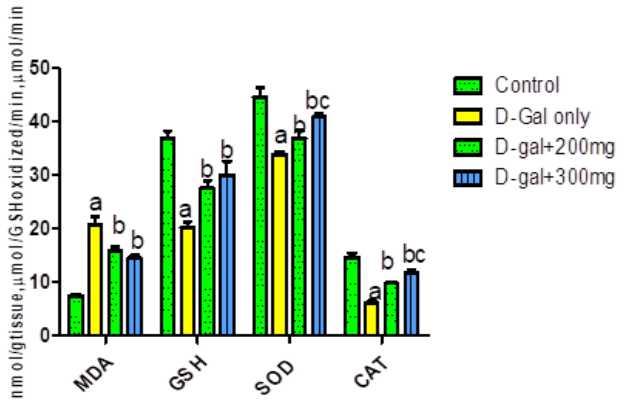

Fig. 1. Effects of Methanolic Leaves Extract of *Vernonia amygdalina* on Malondialdehyde (MDA), Reduced glutathione (GSH), Superoxide dismutase (SOD), and Catalase on Renal tissue of D-galactose-induced Aging in Male Wistar Rats.

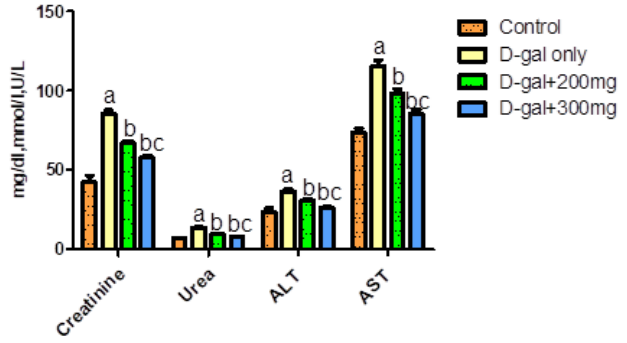

Fig. 2. Effects of Methanolic Leaves Extract of *Vernonia amygdalina* on Alanine Transaminase (ALT), Urea, Aspartate Transaminase (AST), and Creatinine concentration on serum of D-galactose-induced Aging in Male Wistar Rats.

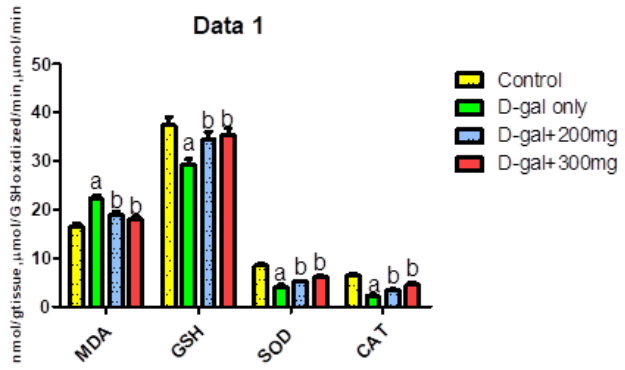

Fig. 3. Effects of Methanolic Leaves Extract of *Vernonia amygdalina* on Malondialdehyde (MDA), Reduced glutathione (GSH), Superoxide dismutase (SOD), and Catalase on Liver tissue of D-galactose-induced Aging in Male Wistar Rats.

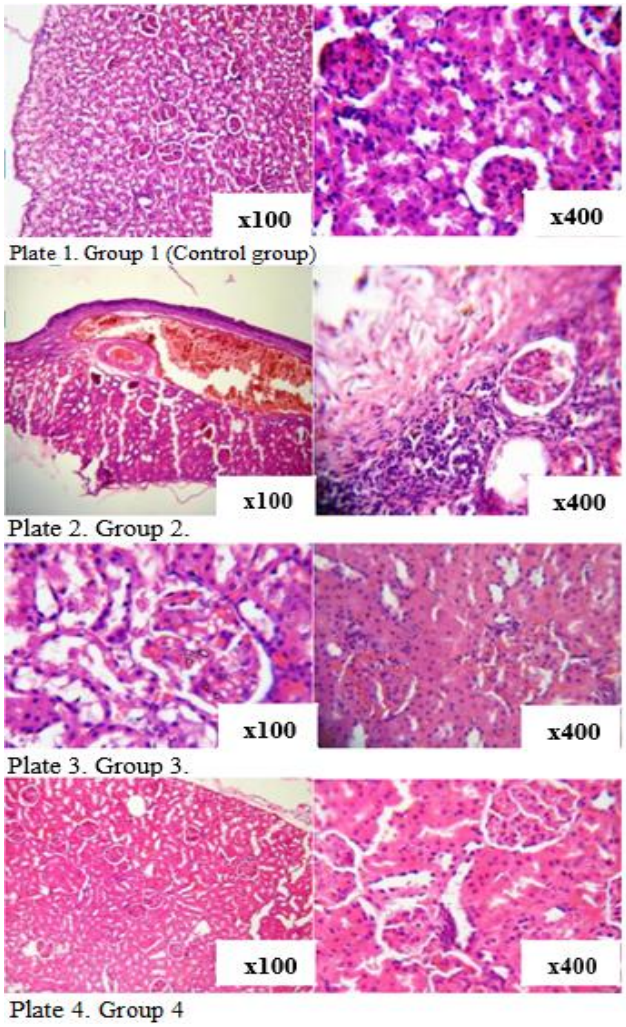

Fig. 4. The effect of *Vernonia amygdalina* on histological changes of D-galactose induced aging rats. The kidney

were removed from each group after treatment with *Vernonia amygdalina*. Section were processed for haematoxylin and eosin (HE) staining. Representative Photomicrograph and Histological image of renal tissues at low and high magnification are shown (x100 and x400).

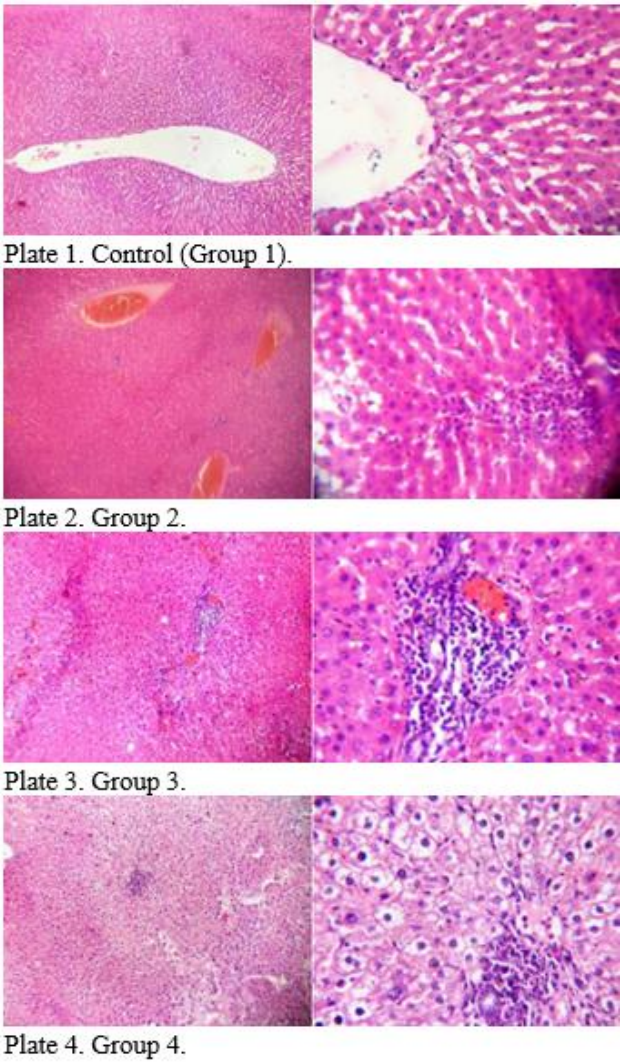

Plate 1. Control (Group 1).

Plate 2. Group 2.

Plate 3. Group 3.

Plate 4. Group 4.

Fig. 5. The effect of *Vernonia amygdalina* on histological changes of D-galactose induced aging rats. The liver were removed from each group after treatment with *Vernonia amygdalina*. Section were processed for haematoxylin and eosin (HE) staining. Representative photomicrograph and histological image of liver tissues at low and high magnification are shown (x100 and x400).

In Table 1, Figure 2 and Table 3 for both renal and hepatic tissues, Reduced glutathione (GSH) concentration of the animals in Group II (D-galactose only treated groups) considerably decreased in concentration compared with those in Group I (Control), the treated groups (Group III & IV) with 200mg/kg body weight and 300mg/kg body weight of *Vernonia amygdalina* extract respectively showed significant increase in the concentration of reduced gluthatione level after treatment when compared with rats in D-galactose only treated groups at $P<0.05$. Elevated level of reduced glutathione occasioned by the extract may be traced to phytochemicals like polyphenols and flavonoids present in the extract also showed possible antioxidant and anti-aging potential of methanolic leave extract of *vernonia amygdalina* and its potential in treatment and management of other oxidative stress diseases.

The results presented in Table 2 D-galactose occasioned a significant increase in AST and ALT activities in group II (D-galactose only) when compared with control groups, but these effects were reversed significantly in groups treated with 200mg/kg bw and 300mg/kg bw extracts respectively in a concentration dependent manner compared with group II (D-galactose only). A significant decrease observed in both ALT and AST activities in serum on treatment with 200mg and 300mg/kg body weight extracts indicates that, *Vernonia amygdalina* extract has potential to ameliorates hepatotoxicity and also suggestive of its possible hepatoprotective potential and this may be traced to phytochemicals present in the methanol leave extract of *Vernonia amygdalina* this also reflects that, the plant extract can counter aging effect occasioned by D-galactose.

Serum Creatinine concentration (Fig. 3) of the rats in Group II (D-galactose only) considerably increased compared to those in Group I (Control), the treated groups (Group III & IV) with 200mg/kg body weight and 300mg/kg body weight of *Vernonia amygdalina* extract respectively showed significant decrease in concentration dependent manner after treatment, when compared with rats in D-galactose only group at $P<0.05$.The obtained result showed that, *Vernonia amygdalina* has properties that can ameliorates oxidative stress mediated aging in kidney which may be traced to flavonoids and some other secondary metabolites in the methanol extract of the plant.

The findings revealed in Figure 4, The urea concentration of the rats in Group II (D-galactose only) considerably increased compared with those in Group I (Control), the treated groups (Groups III &IV) with 200mg/kg body weight and 300mg/kg body weight of *Vernonia amygdalina* extract respectively, showed significant decrease in urea concentration in a dose dependent manner after treatment, when compared with rats in D-galactose only group at $P<0.05$.

Phytochemicals present in methanol extract of *Vernonia amygdalina* or their metabolites, might have elicited a pathway responsible for a significant decrease in concentration of serum urea in the treated groups, this also, indicates the possible protective and ameliorative effects of the extract against renal oxidative stress and D-galactose induced aging.

Table 1. Effects of Methanolic Leaves Extract of *Vernonia amygdalina* on Malondialdehyde (MDA), Reduced glutathione (GSH), Superoxide dismutase (SOD), and Catalase on Renal tissue of D-galactose-induced Aging in Male Wistar Rats.

PARAMETERS	Group A (CONTROL)	Group B (D-galactoseOnly)	Group C (D-galactose+200mg)	Group D (D – gal+300mg)
MDA(nmol/gtissue)	7.42 ± 0.34	20.78 ± 1.50[a]	15.85 ± 0.76[b]	14.43 ± 0.66[b]
GSH(µmol/GSHoxidized/min)	36.88 ± 1.35	20.21 ± 1.09 [a]	27.55 ± 1.45 [b]	29.93 ± 2.63 [b]
SOD(µmol/min)	44.50 ± 1.86	33.85 ± 0.49 [a]	36.83 ± 1.45 [b]	40.85 ± 0.57[bc]
CATALASE(µmol/min)	44.50 ± 1.86	6.22 ± 0.34 [a]	9.87 ± 0.20 [b]	11.87 ± 0.43[bc]

Values are expressed as mean ± SEM, n = 5. Values were considered significant at P<0.05. 'a' represents significant decrease when compared with control and other groups
'b' represents significant increase when compared with D-galactose only group
'c' represents significant increase when compared with D-galactose+200mg/kg group

Table 2. Effects of Methanolic Leaves Extract of *Vernonia amygdalina* on Alanine Transaminase (ALT), Urea, Aspartate Transaminase (AST), and Creatinine concentration on serum of D-galactose-induced Aging in Male Wistar Rats.

PARAMETERS	Group A (CONTROL)	Group B (D-galactose Only)	Group C (D –gal +200mg)	Group D (D – gal+300mg)
Creatinine(mg/dl)	42.24 ± 4.37	85.63 ± 2.09 [a]	66.75 ± 1.67 [b]	57.73 ± 1.55[bc]
Urea (mmol/l)	6.81 ± 0.14	13.70 ± 0.63 [a]	9.25 ± 0.33 [b]	8.03 ± 0.11[bc]
ALT (U/L)	23.48 ± 2.50	36.40 ± 1.51 [a]	30.72 ± 1.09 [b]	26.28 ± 0.73[bc]
AST (U/L)	73.66 ± 2.62	115.40 ± 4.06 [a]	98.56 ± 2.45 [b]	85.40 ± 2.44[bc]

Level of significance was taken at (P<0.05) for 6 rats per group
'a' represents significant increase when compared with control and other groups **'b'** represents significant decrease when compared with D-galactose only group
'c' represents significant decrease when compared with D-galactose+200mg/kg group

Table 3. Effects of Methanolic Leaves Extract of *Vernonia amygdalina* on Malondialdehyde (MDA), reduced glutathione (GSH), Superoxide dismutase (SOD), and Catalase on Liver tissue of D-galactose-induced Aging in Male Wistar Rats.

PARAMETERS	Group A (CONTROL)	Group B (D-galactose Only)	Group C (D–gal +200mg)	Group D (D – gal+300mg)
MDA(nmol/gtissue)	16.58 ± 0.54	22.35 ± 0.66[a]	18.98 ± 0.59[b]	18.10 ± 0.48[b]
GSH(µmol/GSHoxidized/min)	37.40 ± 1.69	29.27 ± 1.16[a]	34.58 ± 1.52[b]	235.42 ± 1.41[b]
SOD(µmol/min)	8.55 ± 0.17	4.14 ± 0.36[a]	5.23 ± 0.13[b]	6.19 ± 0.17[b]
CATALASE(µmol/min)	6.35 ± 0.44	2.27 ± 0.29[a]	3.41 ± 0.21[b]	4.59 ± 0.48[b]

Values are expressed as mean ± SEM, n = 5. Values were considered significant at P<0.05.
'a' represents significant decrease when compared with control and other groups
'b' represents significant increase when compared with D-galactose only group
'c' represents significant increase when compared with D-galactose+200mg/kg group

Interestingly, the photomicrograph and histology of the renal tissues in plates I, II, III and IV corroborated the effectiveness of methanolic leaves extract of *Vernonia amygdalina* in alleviating aging induced by D-galactoseactose. In plate I (control), the photomicrograph revealed normal architecture in glomeruli, capsular spaces, mesengial cells, renal tubules, interstitial space and epithelial arrangement. No pathological lesion observed which is an indication that the cells were healthy. In plate II (D-galactose only), cystic renal tissues with poor architecture was observed, free radicals occasioned by D-galactose caused infiltration of inflammatory cells, loss of glomerular elements, severe dilation and vascular congestion which resulted to aging. Plate III (d- gal +200mg/kg), also showed kidney section with poor architecture, renal cortex showed mesengial hyperplasia that lacks capsular spaces and mesengial cells. There is a moderate infiltration of inflammatory cells compared to severe peri-glomerular infiltration of inflammatory cells observed in plate II (D-galactose only), this moderate infiltration of inflammatory cells and normal lumen observed might have been due to the free radical scavenging potential of the administered methanolic leave extract of *Vernonia amygdalina*. This suggests the potential of the plant extract to alleviate aging occasioned by D-galactose which is traceable to the secondary metabolites like flavonoids in the methanol extract of the plant. Moreover, in plate IV (D-galactose+ 300mg/kg),

normal architecture was observed in renal cortex, glomeruli, capsular spaces, epithelial arrangement mesengial cells, and interstitial space.

Finally, the photomicrograph and histology of the liver tissue in plates I, II, III and IV confirms the anti-aging effect of *Vernonia amygdalina*. The result presented in Plate 1 (Control group) shows photomicrograph of liver section stained by haematoxylin and eosin showing normal architecture as seen in lower magnification (x100), the central venules seen are not congested, the sinusoids appear normal without infiltration of inflammatory cells. The hepatocytes show normal morphology. In contrast, poor architecture as seen in lower magnification (x100), the central venules and portal tracts moderately congested, the portal tract moderately infiltrated by inflammatory cells and focal area of inflammatory cells were observed in the D-galactose only group (Plate 2). These changes were substantially attenuated as revealed by the photomicrograph of liver section of the group that received *Vernonia amygdalina* methanolic extract (Plate 3). However, the improved effects were more notable in the group IV treated with high dose of *Vernonia amygdalina* (Plate 4). The anti-aging effects of *Vernonia amygdalina* appeared to be concentration dependent as the cellular damages caused by free radical accumulation in D-galactose-induced aging were almost restored to the normal cellular architecture. Tannins, Phenols and flavonoids might have been involved in cellular damage repair observed in this plate.

In order to investigate the usage of *Vernnonia amygdalina* in the treatment of different disorders, an extensive literature survey was done in accordance with the study's research goals. With the experimental design followed correctly, biochemical and histology analysis was then carried out to see how exactly *Vernonia amygdalina* can reverse and restore the antioxidant system of the cells following D-galactose induced aging. After conducting the statistical analysis, the level of ALT, AST, UREA, CREATININE and MDA significantly increased while GSH, CATALASE and SOD reduced in the group given D-galactose only. But these biochemical indices were significantly restored in the group that received *Vernonia amygdalina* compared to the group that received D-galactose only. Similarly, the histological damages were restored in the group that received *Vernonia amygdalina* compared to the group treated with D-galactose only. The findings of this study were utilized to establish that *Vernonia amygdalina* confers an anti-aging effect against D-galactose induced aging.

The result revealed that the administration of *Vernonia amygdalina* provides a more efficient and effective way to ameliorate Hepatic aging, Renal aging and other aging-related diseases. The research (Iwalokun *et al*., 2006) confirms the above findings, stating that pre-administration of *Vernonia amygdalina* reduced acetaminophen-induced adverse effects on liver antioxidant status and protein levels in a dose-dependent manner, implying that this plant has antioxidant activity based on free radical scavenging or antioxidant status regulation, tissue regeneration, or protein maintenance.

Aspirin administration in rats resulted in a depletion of the stomach's antioxidant state, as shown by a considerable drop in SOD activity as well as GSH levels (Adefisayo *et al*., 2018). The increasing rise of MDA in the stomach of aspirin-ulcerated rats implies a state of stress and the development of lipid peroxidation owing to depletion of the gastric mucosa's antioxidant system.

However a substantial decrease in MDA concentration coupled with a pronounced increase in SOD and GSH activity in rats treated with MEVA is an obvious indication of the extract's anti-peroxidative and anti-oxidative capacity. Igile *et al*. (1994) pointed out that flavones found in their work are highly desirable components of *Vernonia amygdalina* leaves and demonstrate antioxidant activity.

Increases in serum triglycerides, LDL-cholesterol and serum MDA in diabetic controls have been reported (Nwanjo, 2005) in accordance with previous reports documenting elevated serum triglycerides and lipid peroxide levels in diabetic subjects. It was also reported that aqueous extract administration of *Vernonia amygdalina* leaf significantly reduced triglycerides and suppressed free radical induced cellular damages.

Implications of the study

Researchers and practitioners seeking to discover effective and powerful treatments for hepatic and renal aging will benefit from this study. The following are the study's implications.

- The research on anti-aging therapies (Zhen *et al.*, 2016; Ahangarpour *et al.*, 2017; Wu *et al.*, 2018; Ma *et al.*, 2019) has successfully developed certain treatments in the amelioration of D-galactose induced aging study. However, the present study investigates the anti-aging potency of *Vernonia amygdalina* against D-galactose induced aging. This provides a more effective and novel treatment against oxidative stress-related and aging-related diseases.
- This study investigated the effectiveness and efficiency of *Vernonia amygdalina* to restore the biochemical indices and the antioxidant status of the cell following D-galactose induced aging. The study can thus be beneficial to medicine, pharmacologist, industrialist and government. Researchers may use the results to underta1ke related studies in the aging treatment domain.

- Researchers in the literature (Aydin *et al.*, 2012; Saleh *et al.*, 2019) have mentioned that Renal and Liver aging are clinical health issues of the society. Therefore this study investigated the ameliorative effect of *Vernonia amygdalina* against Renal and Liver aging.

CONCLUSION

This study investigated the anti-aging effect of *Vernonia amygdalina* and its potential to restore efficiently the antioxidant system of the cell in D-galactose induced aging. The study employs evaluation of the biochemical indices and histological study to see how the plant demonstrates its anti-aging effect. Initially, the rats were administered D-galactose followed by the metanolic extract of *Vernonia amygdalina* as experimentally designed. Later, the samples were prepared for histological and biochemical analysis. Further, the study conducted a statistical analysis with the data. The results reveal that *Vernonia amygdalina* is a potent anti-aging agent which ameliorates Renal and Hepatic aging. It is important to notice that the plant greatly restored the oxidative cellular damage caused by D-galactoseactsoe. Accordingly, it is suggested that the use of *Vernonia amygdalina* as an anti-aging agent will have positive impacts on the health issues as regarding Renal and Liver aging nationwide. As a result, this study will have a significant impact on human health and medicines in many nations throughout the world.

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ABBREVIATION

ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), GSH (Glutathione), SOD (Super oxide dismutase), CAT (Catalase), MDA (Malondialdehyde).

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