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Spondias mombin L. Fruit Pulp Prevents D-Galactose-Induced Cognitive Deficits, Oxidative Stress and Neurodegeneration in Wistar Rats' Prefrontal Cortex

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ABSTRACT

D-galactose (D-Gal) administration is known to cause cognitive impairment in animal models of ageing. *Spondias mombin* L. fruit pulp (SM) is known to ameliorate stress-induced conditions caused by oxidative stress. The neuroprotective role of SM against D-Gal-induced stress in Wistar rats was investigated. Forty-two male Wistar rats were divided into six groups (n=7): control group were fed with normal diet; D-Gal group were treated with D-Gal only; Gal + SM5 group were fed with 5% SM (w/w)-containing diet and treated with D-Gal; Gal + SM10 group were fed with 10% SM (w/w)-containing diet and treated with D-Gal; SM 5 group were fed with 5% SM-containing diet; and SM 10 group were fed with 10% SM-containing diet. Control, SM5, and SM10 groups were treated by 0.9% sodium chloride/day (subcutaneous (s.c), 5 days per week) while D-Gal treatment was 250 mg/kg of D-Gal (s.c., 5 days per week). Administration was done for 6 weeks after which behavioural assessments were conducted, followed by biochemical and histopathological analyses of the prefrontal cortex. The administration of SM-containing diet at the two concentrations investigated in this study showed improvements in cognitive impairment caused by the D-Gal compared to the control. Biochemical assays indicated that D-Gal significantly disrupted antioxidant status, proinflammatory and anti-inflammatory cytokines. SM supplementation reversed these effects. Histopathological analysis confirmed that SM5 and SM10 significantly reversed D-Gal-induced cortical alterations. In conclusion, SM attenuated D-Gal-induced cognitive deficits, oxidative stress, and neurodegeneration in Wistar rats.

Keywords

Spondias mombin, Acetylcholinesterase activities, Ageing, Oxidative stress

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INTRODUCTION

Ageing is a natural and inevitable process that gradually diminishes our body's physiological functions, including cognitive abilities (Dharmarajan, 2021). This decline is influenced by factors such as oxidative stress, neuroinflammation, and changes in neurotransmitter activity (Buccellato *et al*., 2021). To better understand these effects, re-

searchers have developed experimental models that simulate human ageing (Wang *et al.*, 2022). One widely used model involves administering D-Galactose (D-Gal), a substance that induces oxidative stress, inflammation, and neurodegeneration, ultimately leading to cognitive impairments in animal studies (Ahmad *et al.*, 2021; Samad *et al.*, 2022).

D-Gal, a reducing sugar, autooxidizes and glycates to produce reactive oxygen species (ROS), leading to oxidative stress (Kumar *et al.*, 2022). As ROS accumulate, they cause cellular damage, impair mitochondrial function, and increase neuronal apoptosis, which are factors that worsen cognitive decline (Sharma *et al.*, 2021). In addition, D-Gal disrupts the balance of neurotransmitters, particularly affecting the cholinergic system, which is essential for learning and memory (Rossato *et al.*, 2024). Because of these effects, D-Gal-induced ageing models have become a valuable tool for exploring neuroprotective agents aimed at mitigating oxidative stress-related cognitive decline (Kuo *et al.*, 2022; Siswanto *et al.*, 2024).

In recent years, natural products and plant-derived compounds have attracted considerable interest as potential therapies for neurodegeneration and age-related cognitive decline (Kuo *et al.*, 2022; Siswanto *et al.*, 2024). One such promising natural remedy is *Spondias mombin* L. fruit, commonly known as yellow mombin. This tropical fruit-bearing plant has been used in traditional medicine for its wide range of health benefits (Moke *et al.*, 2024). Rich in bioactive compounds like flavonoids, polyphenols, and vitamins, yellow mombin exhibits potent antioxidant, anti-inflammatory, and neuroprotective properties (Evuen and Kpomah, 2023). Studies have shown that its fruit pulp can help alleviate stress-induced conditions by modulating oxidative stress markers and enhancing cellular defence mechanisms (de Souza *et al.*, 2023).

This study aims to evaluate if a diet containing *Spondias mombin* fruit pulp (SM) can mitigate the cognitive impairments caused by D-Gal in Wistar rats in light of the increasing interest in natural neuroprotective compounds. Therefore this research investigated the impact of SM fruit pulp supplementation on several parameters, including behavioural performance, antioxidant levels, acetylcholinesterase (AChE) activity, inflammatory cytokines, and histopathological changes in the prefrontal cortex (PFC) of rats induced with D-Gal. By evaluating SM's effect on oxidative stress markers, cholinergic function, and inflammatory cytokines, this study seeks to elucidate its therapeutic potential in addressing age-related neurodegenerative disorders.

The results will contribute to the growing body of evidence supporting the role of natural antioxidants in neuroprotection. If effective, SM could offer a natural, accessible dietary supplement, improving brain health and reducing cognitive decline associated with ageing.

MATERIALS AND METHODS

Plant Collection

Fresh SM fruits were sourced from a farm in Owode Ede, Osun State, and they were identified by Dr. Gideon Okunlola, Department of Plant Biology, Osun State University, Osogbo, and Mr. Abiodun Omole, Herbarium Unit, Obafemi Awolowo University. A voucher specimen was deposited and compared with the reference specimen already in the herbarium of the Obafemi Awolowo University, Ile-Ife, Nigeria.

Experimental Animals

Forty-two male Wistar rats weighing between 150 and 160 g were obtained from Osun State University Animal House, Osogbo, Osun State. The Institutional Animal Care and Use Committee of Osun State University approved the use of animals in this study. The animals were kept in well-ventilated plastic cages under standard laboratory conditions, with ambient humidity (60–70%), temperature (27±2°C), and light (12 h light/dark cycle, on average), respectively. These conditions reflect a naturally regulated environment, which is consistent with local climatic norms and animal care guidelines. The animals were fed with a standard commercial pellet diet (Top Feeds, a product of Premier Feeds Mills Nigeria Ltd, Ibadan, Nigeria) and provided with clean tap water for two weeks of acclimatisation before the experiment.

SM Fruit Preparation

Matured and ripe SM were harvested from a farm in Owode Ede, Osogbo, Osun State. The fruit peels were meticulously removed, and the pulp was extracted from the seed using a sterilised stainless steel knife. The extracted pulp was then subjected to freeze-drying. The dried materials were used to prepare a 5% w/w and 10% w/w SM diet with the standard commercial pellet.

Experimental Design

Forty-two male Wistar rats were divided into six groups based on their weights, consisting of seven rats per group. The control group were rats fed with a normal diet and administered with normal saline. D-Gal group rats were administered with D-Gal procured from Sigma Aldrich, Canada. D-Gal + 5SM group were rats treated with the D-Gal and 5% w/w SM. D-Gal + 10SM group rats were also induced with D-Gal and treated with 10% w/w SM. 5SM group were rats treated with 5% w/w SM. The SM10 group were rats treated with 10% w/w SM. Normal saline or D-Gal at 250 mg/kg body weight were administered subcutaneously five times per week, and rats were exposed to standard feed or SM freely and orally throughout. The treatments, consisting of daily exposure to either standard feed or SM, along with concurrent subcutaneous administration of D-Gal for five successive days each week, were carried out over a period of six weeks. After the 6th week, the behavioural test was conducted on the animals.

Behavioural Assay

Step-Down Latency

Step-down latency in the inhibitory avoidance test was carried out using the method of Izquierdo and Dias (1983) as described by Osuntokun *et al*. (2021). Each rat was placed in the shock-free zone and allowed to explore the chamber for 10 min. On stepping down onto the grid floor, the animal received an electric shock (20 V) through the grid floor. Two hours later, this procedure was repeated, and the time taken for each rat to step down was measured in

seconds. This is known as the step-down latency, and this constitutes the training process. Following the training session, step-down latency by each rat was determined after 24 h of the training session. A prolongation or shortening of step-down latency was used as a parameter of learning and memory.

Novel Object Recognition Task
This was done to determine working and spatial memory in animals using the method of Ennaceur (2010) described by Osuntokun *et al*. (2021). Three days before testing, rats acclimated to two identical objects for 5 min before returning to their home cages. After 30 min, a probe test introduced a novel object, and exploration time was recorded as a memory index by a blinded experimenter.

Open Field Test
This was achieved by determining the total number of horizontal and vertical lines crossed by each rat in a wooden box measuring 1m long × 1m wide × 1m high according to the method of Seibenhener and Wooten (2015) as described by Osuntokun *et al*. (2021).

Collection and Homogenisation of PFC
The rats were euthanised through cervical dislocation 24 hours after the behavioural assay. The brain was excised (representatives of each group were kept for histological study), and the PFC was removed from the brain and rinsed in a solution of normal saline, weighed, and homogenised in phosphate buffer solution (0.1M, pH = 7.2). The resulting homogenate was then centrifuged for 10 minutes at 10,000 rpm and at the temperature of 4°C to obtain the supernatant fraction, which was then used to carry out the biochemical assays.

Histological Study
The excised PFC were thereafter post-fixed in 4% paraformaldehyde (PFA) for 48 hours and then processed for qualitative studies. PFA-fixed tissue sections were stained using haematoxylin and eosin (H and E) staining techniques.

Biochemical Assays
Antioxidant assays
Antioxidant assays were carried out on the supernatants from the tissue of the PFC. The extent of lipid peroxidation in the PFC was determined by measuring the amount of malondialdehyde (MDA) following a spectrophotometric method described by Ohkawa *et al*. (1979). Glutathione (GSH) levels were measured using Ellman's (1959) method, based on the reaction with Ellman's reagent and phosphate buffer, with absorbance recorded at 412 nm (Vuolo *et al.*, 2022). Catalase activity was estimated by using the method of Jeje *et al*. (2024) by measuring the decrease in absorbance as the enzyme breaks down hydrogen peroxide at 240 nm. The activity was calculated using an extinction coefficient of 0.0436 $mm^{-1}cm^{-1}$. The glutathione-s-transferase activity was determined by the method of Habig *et al*. (1974) using 1-chloro-2,4-dinitrobenzene as a substrate. Superoxide dismutase (SOD) activity was measured by the McCord and Fridovich (1969) method, based on the enzyme's catalysis of superoxide dismutation to oxygen and hydrogen peroxide, with spectrophotometric quantification.

ELISA Analysis for Cytokines (Tumour Necrosis Factor Alpha, Interleukin 10, and Interleukin-1β) Levels
The pro-inflammatory cytokines (tumour necrosis factor alpha**,** TNF-α and interleukin 1β**,** IL-1β) levels and anti-inflammatory cytokines (interleukin 10, IL-10) were measured in PFC by using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions; they were products of Elabscience, USA.

Estimation of AChE Activity
The supernatants from the tissue of the PFC were evaluated for the activities of AChE using the Ellman *et al*. (1961) method, based on the formation of a yellow anion measured at 412 nm. The reaction involved K^+-phosphate buffer, 5,5'-dithiobis-(2-nitrobenzoic acid), and acetylthiocholine iodide, with enzyme activity expressed in units/mg protein.

Data Analysis
The values were expressed as mean ± standard error mean (SEM). The mean differences in each group were analysed by one-way ANOVA using GraphPad Prism 8.0.1, followed by Turkey post hoc tests at a probability level of 0.5.

RESULTS

Effects of SM on Behavioural Patterns of Male Wistar Rats Treated with D-Gal
Step-Down Latency
Administration of D-Gal significantly ($p < 0.05$) decreased the step-down latency, a marker of long-term memory index compared with the control. However, treatment with SM5 or SM10 significantly increased ($p < 0.05$) the step-down latency when compared with the D-Gal group (Fig. 1A).

Novel Object Recognition
There was a non-significant decrease ($p > 0.05$) in the percentage novel object recognition following the D-Gal treatment compared with the control group. However, novel object recognition increased significantly ($p < 0.05$) in the D-Gal co-treated with 5SM and 10SM diets and the SM alone treatment group (Fig. 1B).

Open Field Test
In D-Gal only treated animals, the number of lines crossed was significantly reduced ($p < 0.05$) compared with normal control. However, administration of SM10 significantly increase ($p<0.05$) the number of lines crossed when compared with D-Gal group. Overall, SM diets improved the exploratory activities (Fig. 1C).

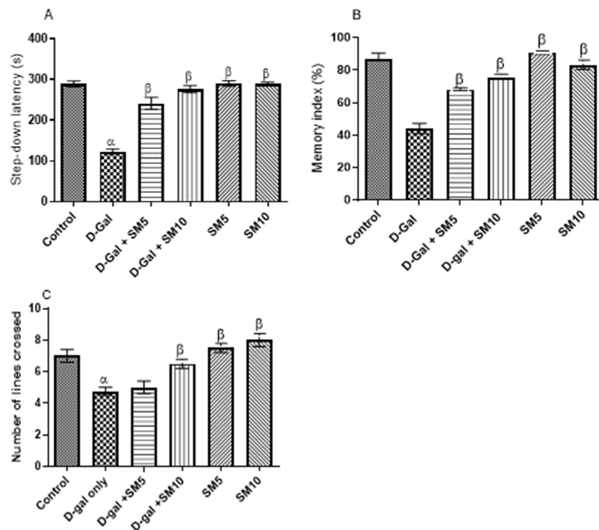

Fig. 1: Effects of SM diet on long-term memory (A), novel object recognition (B), and exploratory and locomotor in the open field test activities (C) of male Wistar rats treated with D-Gal. Values are expressed as mean ± SEM (n=7). α - significant compared to the control group ($p < 0.05$); β - significant compared to the D-Gal group ($p < 0.05$).

Effects of SM on Antioxidant Parameters in the PFC of Wistar Rats Treated with D-Gal

The SM-supplemented diet significantly ($p < 0.05$) mitigated the increase in PFC MDA level occasioned by D-Gal treatment, indicating enhanced lipid peroxidation. SM5 and SM10 significantly reduced ($p < 0.05$) PFC MDA levels compared to D-Gal treated rats (Fig. 2A).

The PFC GSH level was significantly decreased in the D-Gal group compared to the control group ($p < 0.05$), indicating oxidative stress. Administration of SM5 and SM10 to Administration of SM5 and SM10 to D-Gal-treated animals resulted to non-significant increase ($p > 0.05$) in PFC GSH concentrations relative to the D-Gal-treated group, with values remaining significantly lower than the control ($p < 0.05$). However, the SM5 and SM10 groups showed a significant increase in PFC GSH levels compared to both the D-Gal group ($p < 0.05$) and the control group ($p < 0.05$), suggesting a strong antioxidative effect (Fig. 2B).

The PFC SOD activity was significantly decreased in the D-Gal group compared to the control group ($p < 0.05$). Administration of SM5 or SM10 to D-Gal-treated animals induced a significant increase ($p < 0.05$) in PFC GSH concentrations relative to the D-Gal-treated group, which remained significantly lower than that of the control group ($p < 0.05$) but was significantly higher than that of the D-Gal group ($p < 0.05$). Meanwhile, the SM5 and SM10 groups showed SOD activities that were slightly higher than the control group ($p > 0.05$) (Fig. 2C).

Catalase activity was significantly reduced in the D-Gal group compared to the control group ($p < 0.05$). Consistent with other findings, administering SM10 to D-Gal–treated animals resulted in a significant increase ($p < 0.05$) in catalase activity compared with D-Gal treated group, also a non-significant increase ($p>0.05$) in catalase levels compared to the D-Gal-only treated animals were observed with SM5 treatment (Fig. 2D).

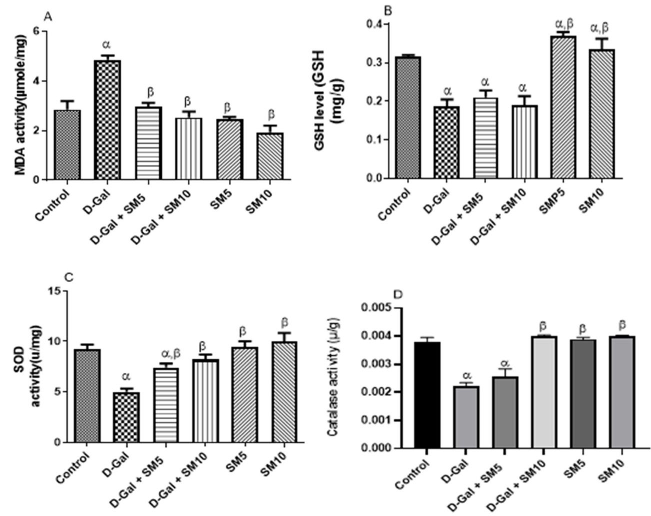

Fig. 2: Effects of SM on lipid peroxidation (A), glutathione level (B), superoxide dismutase activity (C), and catalase activity (D) of male Wistar rats treated with D-Gal. Values are expressed as mean ± SEM (n=7). α - significant compared to the control group ($p < 0.05$); β - significant compared to the D-Gal group ($p < 0.05$).

Effects of SM on the AChE in the PFC of Wistar Rats Treated with D-Gal

The AChE activity was significantly reduced in the D-Gal group compared to the control group ($p < 0.05$). Administration of SM5 and SM10 to D-Gal-treated animals resulted in a non-significant increase ($p > 0.05$) in AChE activity compared to the D-Gal treated group, though the level of AChE in D-Gal+SM5 group remained significantly lower ($p<0.05$) than the control while the level of AChE in D-Gal+SM10 group remained non-significantly lower ($p>0.05$) when compared with the control group. Conversely, the SM5 and SM10 groups exhibited significantly higher AChE activity compared to the D-Gal group ($p < 0.05$), indicating a potential protective effect (Fig. 3).

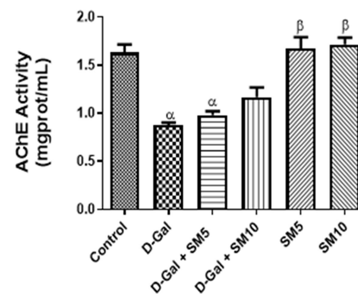

Fig. 3: Effects of SM on acetylcholine activity of male Wistar rats treated with D-Gal. Values are expressed as mean ± SEM (n=7). α - significant compared to the control group ($p < 0.05$); β - significant compared to the D-Gal group ($p < 0.05$).

Effects of SM on the Pro-Inflammatory Cytokines in the PFC of Wistar Rats Treated with D-Gal

Pro-inflammatory cytokine

Interleukin-1β levels were significantly elevated in the D-Gal only group compared to the control group ($p < 0.05$). Co-treatment with either SM5 or SM10 in D-Gal–treated animals resulted in significant increase ($p < 0.05$) in IL-1β

levels when compared with the control group. However, co-treatment with SM5 and SM10 groups showed a mild reduction in IL-1β levels compared to the D-Gal only group ($p < 0.05$) (Fig, 4A).

TNF-α levels were significant increase ($p < 0.05$) in the D-Gal only group compared to the control group ($p < 0.05$). Co-treatment with either SM5 or SM10 in D-Gal–treated animals significantly reduced TNF-α levels relative to the D-Gal only group ($p < 0.05$), but these values were significantly increased ($p < 0.05$) when compared to control group ($p < 0.05$). Meanwhile, SM5 and SM10 alone did not differ from the control but were significantly lower than the D-Gal only group ($p < 0.05$) (Fig. 4B).

Anti-Inflammatory Cytokine

The IL-10 levels were significantly higher in the D-Gal + SM5 and D-Gal + SM10 groups compared to both the Control group ($p < 0.05$) and the D-Gal only group ($p < 0.05$). In addition, the SM5 and SM10-administered groups exhibited significantly higher IL-10 levels than the D-Gal group ($p < 0.05$), although they did not differ significantly from the control (Fig. 4C).

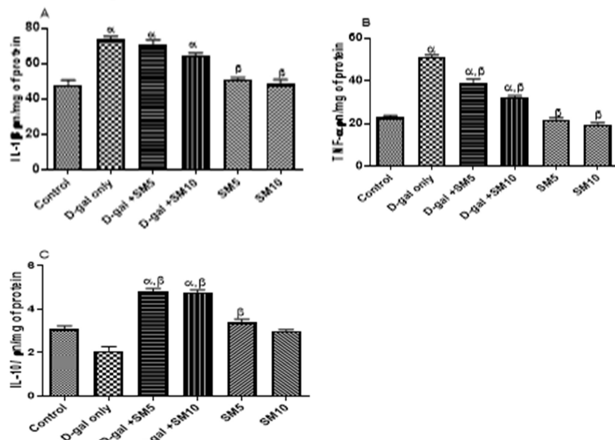

Fig 4: Effects of SM on interleukin-1β level (A), TNF-α level (B) and IL-10 level of male Wistar rats treated with D-Gal. Values are expressed as mean ± SEM (n = 7). α - significant compared to the control group ($p < 0.05$); β - significant compared to the D-Gal group ($p < 0.05$).

Histology Studies

Figure 5 shows the representative micrographs of the PFC in male Wistar rats across the study groups. The control, SM5, and SM10 treatments exhibited normal histological features of the PFC. These groups consist of large pyramidal and granule neurons, with pyramidal cells distinguished by elongated axons that extend from the soma to establish connections with adjacent neurons within the neuropil. Apical and basal dendrites extend from the well-delineated soma of the pyramidal neurons in these groups. Perineural space surrounding these cells appeared intact, with intact nuclear and cytoplasmic content.

In contrast, D-Gal treatment induced notable degenerative alterations in the PFC. These changes were marked by clustered pyknotic pyramidal neurons exhibiting fragmented cytoplasm and condensed nuclei within the soma (indicated by red arrows). Perineural spaces were observed encasing degenerating neurons, while axons and dendrites appear markedly reduced in proximity to neuronal bodies. Additionally, this group experienced a notable reduction in the overall neuronal population.

Furthermore, rats exposed to D-Gal + SM5 and D-Gal + SM10 showed mild alterations in the microarchitecture of the PFC, but the pyramidal neurons appeared larger with considerably longer axons, and their nuclei are mostly unfragmented with appreciable cytoplasmic as well as nuclear contents.

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DISCUSSION

The PFC is a critical neural substrate underpinning higher-order cognitive processes such as memory encoding, decision-making, and executive control (Fuster, 2017). This region is among the first to exhibit age-related neurodegenerative changes, largely due to its heightened susceptibility to oxidative stress and inflammatory insults (Morrison and Baxter, 2012).

In this study, we investigated the impact of D-Gal-induced ageing on the PFC of male Wistar rats. D-Gal is known to elevate the production of ROS, which in turn oxidise essential cellular macromolecules and impair mitochondrial function. This oxidative insult activates a cascade of downstream signalling pathways, notably the induction of stress-

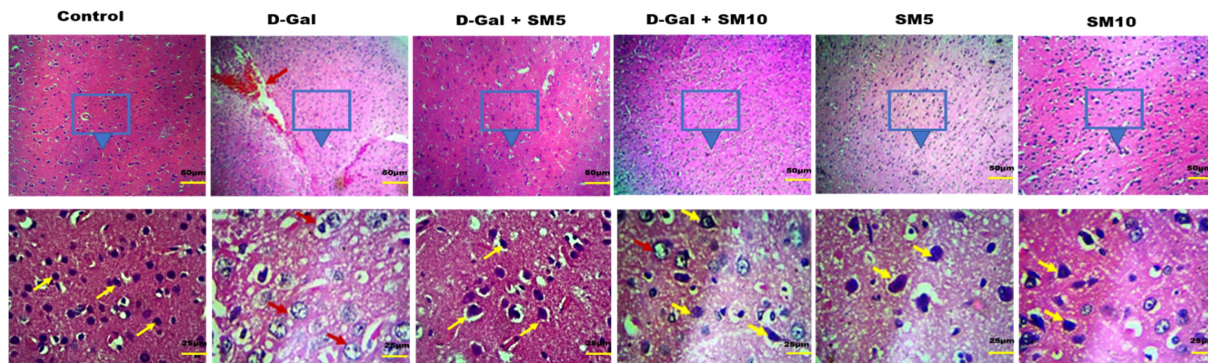

Fig. 5: Photomicrographs depicting the cytoarchitectural organisation of the PFC (H and E stain) at low (50 μm) and high (25 μm) magnifications, illustrating micromorphological characteristics in male Wistar rats across experimental groups. The blue box emphasises the external pyramidal layer (III) and internal granular layer (IV) at 25 μm. Yellow arrows indicate mildly intact and morphologically preserved cells, whereas red arrows highlight degenerating cells exhibiting cytoplasmic and nuclear material depletion.

responsive transcription factors that further exacerbate neurodegenerative processes (Mursal *et al.*, 2024). Significantly, our findings demonstrate that treatment with SM effectively counteracts these deleterious effects. The observed alterations in oxidative stress markers, inflammatory responses, and cognitive function provide valuable insights into the molecular mechanisms driving age-related deterioration in the PFC.

In this study, we investigated the impact of D-Gal-induced ageing on the PFC of male Wistar rats. D-Gal is known to elevate the production of ROS, which in turn oxidise essential cellular macromolecules and impair mitochondrial function. This oxidative insult activates a cascade of downstream signalling pathways, notably the induction of stress-responsive transcription factors that further exacerbate neurodegenerative processes (Mursal *et al.*, 2024). Significantly, our findings demonstrate that treatment with SM effectively counteracts these deleterious effects. The observed alterations in oxidative stress markers, inflammatory responses, and cognitive function provide valuable insights into the molecular mechanisms driving age-related deterioration in the PFC.

The pronounced reduction in GSH levels observed in the D-Gal-treated group relative to controls is indicative of oxidative stress, a key marker of age-associated cellular damage (Azman and Zakaria, 2019). GSH, a pivotal intracellular antioxidant, detoxifies ROS, and its depletion signifies a disturbed redox balance in the PFC. In parallel, significantly elevated MDA levels, a lipid peroxidation byproduct, further corroborate the presence of oxidative injury, in alignment with previous findings on ROS-mediated neuronal damage (Mursal *et al.*, 2024).

Co-treatment with SM extracts (SM5 and SM10) resulted in a partial, albeit incomplete, restoration of GSH levels. This observation suggests that SM can bolster the antioxidant defences within the PFC, although it may not entirely reverse extensive oxidative damage. Notably, both the SM5 and SM10 groups exhibited significant enhancement in GSH levels compared to the D-Gal and control groups, implying an upregulation of oxidative defence mechanisms potentially attributable to the polyphenolic constituents of SM. These bioactive compounds are known to scavenge free radicals and stimulate antioxidant enzyme activities (Cabral *et al.*, 2016).

Typically, oxidative stress upregulates critical enzymes such as SOD and catalase, which serve to mitigate further oxidative damage. However, the marked decline in SOD and catalase activities in the D-Gal group reflects a compromised endogenous antioxidant defence system. Although SM5 and SM10 treatments did not fully restore SOD activity to control levels, both significantly increased SOD and catalase activities compared to the D-Gal group, suggesting that SM partially recovers the enzymatic antioxidant capacity in the PFC.

Additionally, the PFC plays a crucial role in cholinergic neurotransmission, which is vital for cognitive functions such as attention and memory consolidation (Morrison and Baxter, 2012). The observed reduction in AChE activity in the D-Gal group is indicative of cholinergic impairment, consistent with the established link between oxidative stress and cognitive decline (Mukherjee *et al.*, 2007). Although SM5 and SM10 treatments only partially ameliorated the decline in AChE activity, the significant increase in AChE activity in the co-treatment groups relative to the D-Gal group underscores SM potential modulatory effect on the cholinergic system in the PFC, which may contribute to improved cognitive outcomes.

Beyond oxidative stress, inflammation is a critical contributor to the cognitive decline observed with ageing (Ahmad *et al.*, 2021). Our study demonstrated that treatment with D-Gal led to elevated levels of pro-inflammatory cytokines, including TNF-α and IL-1β, indicative of a chronic inflammatory state. Such increases in inflammatory markers are well-documented consequences of oxidative stress, which in turn exacerbate neuronal damage (Magrone *et al.*, 2019). Notably, co-administration of SM resulted in a significant reduction of TNF-α and IL-1β levels, underscoring its potent anti-inflammatory properties. Furthermore, the D-Gal + SM5 and D-Gal + SM10 groups exhibited significantly elevated levels of interleukin-10 (IL-10), a cytokine associated with immunosuppressive responses. This cytokine modulation suggests that SM may help rebalance the inflammatory milieu in the ageing PFC, potentially by inhibiting the nuclear factor-kappa B (NF-κB) signalling pathway, a key mediator of inflammatory responses (Magrone *et al.*, 2019).

In D-Gal-treated rats, significant cognitive deficits and behavioural changes were observed, including reduced step-down latency, impaired novel object recognition, and decreased exploration in the open field. These findings highlight the detrimental impact of oxidative stress, inflammation, and neurotransmitter dysfunction on PFC-dependent functions. Our results are in line with previous studies linking these degenerative processes to long-term memory impairment (Mursal *et al.*, 2024). Notably, supplementation with SM significantly mitigated these cognitive and behavioural deficits, suggesting its protective efficacy. The observed improvements in novel object recognition, memory retention, and exploratory activity could mean that SM preserves PFC-dependent functions through mechanisms that include antioxidant recovery, inflammation modulation, and cholinergic restoration.

Histological analysis of the cortex corroborated these findings. The control and SM-treated groups exhibited normal cytoarchitecture with intact pyramidal and granule neurons, while the D-Gal group showed pronounced degenerative changes, including pyknotic neurons with fragmented cytoplasm and condensed nuclei. The attenuation of these histopathological alterations in the SM-treated groups provides further evidence that SM protects against D-Gal–induced neurodegeneration in the PFC (Aon-Im *et al.*, 2024).

Conclusion

This study provides data that oxidative stress and inflammation are central pathophysiological drivers of PFC degeneration in the context of D-Gal-induced ageing. Our findings affirmed the vulnerability of the PFC to redox imbalance and inflammatory insults, which collectively compromise neuronal integrity, synaptic function, and ultimately, cognitive performance. The administration of SM extract demonstrated significant neuroprotective effects, mitigating biochemical, behavioural, and histopathological markers of ageing-induced neurodegeneration. Collectively, these

findings underscore SM's multifaceted neuroprotective capacity in counteracting age-related PFC deterioration. The extract not only attenuated oxidative and inflammatory cascades but also preserved neurobehavioural function and cellular architecture. These protective effects appeared to be dose responsive, further validating the therapeutic potential of SM in modulating neurodegenerative processes.

Future research should delve deeper into the specific molecular targets of SM, with particular emphasis on transcriptional regulators such as nuclear factor erythroid 2–related factor 2 (Nrf2) and NF-κB, to better elucidate the extract's mechanistic pathways. Additionally, translational studies exploring the pharmacokinetics and clinical efficacy of SM in human models of age-associated neurodegeneration are warranted. Such investigations will be critical to determining its viability as a phytotherapeutic candidate in the management of neurocognitive disorders linked to PFC dysfunction.

DECLARATION

Acknowledgements

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This work was self-funded.

Conflict of Interest

None declared.

Ethical Approval

Ethical approval for the study was obtained from the Health Research Ethics Committee (HREC) of the College of Health Sciences, Osun State University, Osogbo, Nigeria (Approved protocol no: UNIOSUNHREC/B/ 2024/016).

Consent to Participate and Publish Data

Not applicable.

Authors' Contribution

TGA and FMO - Conception, design and supervision of the experiment; TGA, FMO, OST, OSO, OOO, AAB, OSA, KIA and MTA - Experimentation and data collection; TGA, OST and OSO - Data analysis and interpretation; TGA, OST and OSO - Drafting of the manuscript; AAB and JOF - Critical revision of the manuscript.

REFERENCES

Ahmad, S., Khan, A., Ali, W., Jo, M. H., Park, J., Ikram, M., et al. (2021). Fisetin rescues the mice brains against D-Galactose-induced oxidative stress, neuroinflammation and memory impairment. Front Pharmacol 12, 612078. doi: 10.3389/fphar.2021.612078

Aon-Im, P., Monthakantirat, O., Daodee, S., Chulikhit, Y., Sriya, N., Boonyarat, C., et al. (2024). Evaluation of the impact of Alternanthera philoxeroides (Mart.) Griseb. Extract on memory impairment in D-galactose-induced brain aging in mice through its effects on antioxidant enzymes, neuroin-flammation, and telomere shortening. *Molecules* 29(2), 503. doi: 10.3390/molecules29020503

Azman, K. F., and Zakaria, R. (2019). D-Galactose-induced accelerated ageing model: an overview. *Biogerontology* 20(6), 763-782. doi:.10.1007/s10522-019-09837-y

Buccellato, F. R., D'Anca, M., Fenoglio, C., Scarpini, E., and Galimberti, D. (2021). Role of oxidative damage in alzheimer's disease and neurodegeneration: From pathogenic mechanisms to biomarker discovery. *Antioxidants* 10(9), 1353. doi: 10.3390/antiox10091353

Cabral, B., Siqueira, E. M., Bitencourt, M. A., Lima, M. C., Lima, A. K., Ortmann, C. F., et al. (2016). Phytochemical study and anti-inflammatory and antioxidant potential of Spondias mombin leaves. *Revista Brasileira de Farmacognosia* 26, 304-311. doi: 10.1016/j.bjp.2016.02.002

de Souza, V. R., Lima, T. P. B., Bedê, T. P., Faria, S. B. A., Alves, R., Louzada, A., et al. (2023). Murici (Byrsonima crassifolia (L.) Kunth and verbascifolia (L.)) and tapereba (Spondias mombin) improve hepatic and inflammatory biomarkers in high-fat-diet rats. *Foods* 12(2), 255. doi: 10.3390/foods12020255

Dharmarajan, T. (2021). Physiology of ageing. Geriatric *Gastroenterology* 101-153. doi: 10.1007/978-3-030-30192-7_5

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Arch Biochem Biophys* 82(1), 70-77. doi: 10.1016/0003-9861(59)90090-6

Ellman, G. L., Courtney, K. D., Andres Jr, V., and Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7(2), 88-95. doi: 10.1016/0006-2952(61)90145-9

Ennaceur, A. (2010). One-trial object recognition in rats and mice: methodological and theoretical issues. *Behav Brain Res* 215(2), 244-254. doi: 10.1016/j.bbr.2009.12.036

Evuen, U., and Kpomah, E. D. (2023). Comparative impact of solvent extracts of Spondias mombin leaves on in-vitro antioxidant and acetylcholinesterase inhibitory activities (2023). *Asian J Res Biochem* 12(3), 20-29. DOI: 10.9734/ajrb/2023/v12i3236

Fuster, J. (2017). Prefrontal cortex in decision-making: The perception-action cycle. Decision neuroscience. Elsevier, pp. 95-105. doi: 10.1016/B978-0-12-805308-9.00008-7

Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *J Biol Chem* 249(22), 7130-7139. doi: 10.1016/S0021-9258(19)42083-8

Izquierdo, I., and Dias, R. D. (1983). Effect of ACTH, epinephrine, β-endorphin, naloxone, and of the combination of naloxone or β-endorphin with ACTH or epinephrine on memory consolidation. *Psychoneuroendocrinology*, 8(1), 81-87. doi: 10.1016/0306-4530(83)90043-4

Jeje, S. O., Omayone, T. P., Oyeleye, I. S., Adegoke, A. M., and Adeagbo, A. S. (2024) Protective role of vitamin supplementation against maternal stress-induced alterations in cardiovascular indices and cardiac oxidative stress in offspring of Wistar rats*. FUTA Journal of Life Sciences* 4(1), 39-52.

Kumar, H., Bhardwaj, K., Valko, M., Alomar, S. Y., Alwasel, S. H., Cruz-Martins, N., Dhanjal, D. S., Singh, R., Kuča, K., and Verma, R. (2022). Antioxidative potential of Lactobacillus sp. in ameliorating D-Galactose-induced age-

ing. *Appl Microbiol Biotechnol* 106(13), 4831-4843. doi: 10.1007/s00253-022-12041-7

Kuo, H.-C., Tong, S.-Y., Chao, M.-W., and Tseng, C.-Y. (2022). Ganoderma tsugae prevents cognitive impairment and attenuates oxidative damage in D-Galactose-induced ageing in the rat brain. *PLoS One* 17(4), e0266331. doi: 10.1371/journal.pone.0266331

Magrone, T., Magrone, M., Russo, M. A., and Jirillo, E. (2019). Recent advances on the anti-inflammatory and antioxidant properties of red grape polyphenols: in vitro and in vivo studies. *Antioxidants* 9(1), 35. doi: 10.3390/antiox9010035

McCord, J. M., and Fridovich, I. (1969). Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J boil Chem* 244(22), 6049-6055. doi: 10.1016/S0021-9258(18)63504-5

Moke, E. G., Edje, E. K., Daubry, T. M., Nwogueze, B. C., Ataikiru, O. M., Umukoro, E. K., et al. (2024). Phytopharmacological Activities of Spondias mombinLinn: A Review: *Tropical Journal of Phytochemistry and Pharmaceutical Sciences* 3(1), 117-123. doi:10.26538/tjpps/v3i1.1

Morrison, J. H., and Baxter, M. G. (2012). The ageing cortical synapse: hallmarks and implications for cognitive decline. *Nat Rev Neurosci* 13(4), 240-250. doi: 10.1038/nrn3200

Mukherjee, P. K., Kumar, V., and Houghton, P. J. (2007). Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. *Phytother Res* 21(12), 1142-1145. doi: 10.1002/ptr.2224

Mursal, M., Kumar, A., Hasan, S. M., Hussain, S., Singh, K., Kushwaha, S. P., et al (2024). Role of natural bioactive compounds in the management of neurodegenerative disorders. *Intelligent Pharmacy* 2(1), 102-113. doi: 10.1016/j.ipha.2023.09.006

Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95(2), 351-358. doi: 10.1016/0003-2697(79)90738-3

Osuntokun, O. S., Aderoju, M. O., Adebisi, I. E., Abayomi, T. A., Tokunbo, O. S., and Olayiwola, G. (2021). Phenytoin-levetiracetam adjunctive treatment-induced neurotoxicity and deregulation of cholinergic neurotransmission with evidence of neurocognitive impairment in male Wistar rats. *Alex J Med* 57(1), 194-204. doi: 10.1080/20905068.2021.1948157

Rossato, D. R., Rosa, J. L., Fontoura, M. B., de Souza, L. E., de Almeida, T. M., Kudrna, K. B., et al. (2024). Ferulic acid-loaded nanostructure maintains brain levels of ACh, glutamate, and GABA and ameliorates anxiety and memory impairments induced by the D-galactose ageing process in rats. *Neurochem Res*, 49(12), 3383-3395. doi: 10.1007/s11064-024-04248-z

Samad, N., Hafeez, F., and Imran, I. (2022). D-Galactose induced dysfunction in the mice hippocampus and the possible antioxidant and neuromodulatory effects of selenium. *Environmental Science and Pollution Research* 29(4), 5718-5735. doi: 10.1007/s11356-021-16048-x

Seibenhener, M. L., and Wooten, M. C. (2015). Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *J Vis Exp* (96), 52434. doi: 10.3791/52434-v

Sharma, C., Kim, S., Nam, Y., Jung, U. J., and Kim, S. R. (2021). Mitochondrial dysfunction as a driver of cognitive impairment in Alzheimer's disease. *Int J Mol Sci* 22(9), 4850. doi: 10.3390/ijms22094850

Siswanto, F. M., Wijaya, I. M. T., Handayani, M. D. N., Dewi, R., Ekowati, A. L., and Manalu, J. L. (2024). Extract of Angelica keiskei Leaves Attenuates Spatial Memory Impairment on the D-g model of brain ageing in mice. *Biomed Pharmacol J* 17(3), 1563-1573.doi: 10.13005/bpj/2965

Vuolo, M. M., da Silva-Maia, J. K., and Batista, Â. G. (2022). The GSH colorimetric method as measurement of antioxidant status in serum and rodent tissues. In Basic Protocols in Foods and Nutrition. Springer. pp. 187-194. doi: 10.1007/978-1-0716-2345-9_12

Wang, K., Liu, H., Hu, Q., Wang, L., Liu, J., Zheng, Z., et al.. (2022). Epigenetic regulation of ageing: implications for interventions of ageing and diseases. Signal Transduct Target Ther 7(1), 374. doi: 10.1038/s41392-021-00773-3

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