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ARTICLE

Sweet orange (Citrus sinensis) juice mitigates some pathophysiological disorders in D-galactose-induced aging in rats

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Abstract
Aging is associated with several pathophysiological alterations that cause a decline in the functions and physiological activities of an organism. *Citrus sinensis* (Sweet orange) is the most consumed citrus fruit for nutritional and pharmacological purposes. This study investigated the anti-aging effects of *C. sinensis* juice in D-galactose-induced aging rats. Five female Wistar rats each were randomly assigned to four different groups, namely: control (distilled water), aging (D-galactose), orange juice (D-galactose and sweet orange), and the standard (D-galactose and quercetin). Antioxidant enzymes in the heart, liver, kidney, and brain were monitored, while lipid profile was estimated in the blood. Serum inflammatory markers and female sex hormones were also monitored using the ELISA kits. Administration of D-galactose increased the level of LDL-Cholesterol in the model group, however, administration of *C. sinensis* significantly reduced it in the treated group. Superoxide dismutase (SOD) and catalase (CAT) activities were significantly increased in the tissues of the treated animals ($p < 0.05$). The juice has no significant effect on estradiol and luteinizing hormone. *C. sinensis* juice could mitigate some pathophysiological alterations associated with aging processes by improving tissue antioxidant capacity, reducing inflammation, as well as dyslipidemia.

Keywords: Aging, oxidative stress, inflammation, sex hormones, sweet orange, *citrus sinensis*

1.0 Introduction

Citrus (*Rutaceae*) is a plant class comprising different fruits widely consumed worldwide as fresh produce and juice for nutritional and refreshment purposes (Lin et al., 2019). Various types of citrus fruits, including mandarins (*Citrus reticulata*), sweet oranges (*Citrus sinensis*), pomelos (*Citrus maxima*), lemon (*Citrus limon*), lime (*Citrus aurantifolia*), etc., which are also of great economic importance based on their abundance in different regions are known (Lv et al., 2015). Different parts of these fruits are exploited for various therapeutic purposes based on their rich phytochemical constituents (Addi et al., 2021). Their juices have been consumed as functional foods, potentially treating many diet-related ailments like kidney stones, dyslipidemia, and inflammatory processes (Lin et al., 2019). *Citrus sinensis* is the most popular, most cultivated, and one of the most consumed citrus fruits. About 70% of total citrus fruits cultivated yearly are *C. sinensis* (Zahr et al., 2023).

The consumption of fruits such as sweet oranges is usually studied for health purposes because of its rich phytonutrients (Okwu, 2008). *Citrus sinensis* juice is a major sourced fruit juice globally, for nutritional and pharmacological benefits (Gupta et al., 2021). It has been reported that regular consumption of orange juice reduces oxidative stress, inflammation, and bad fats (Ribeiro et al., 2021). Moreover, orange juice could provide therapeutic phytochemicals, such as phenolic compounds, carotenoids, and vitamin C, which account for its antioxidant properties (Arilla et al., 2021). One of the major physiological conditions responsible for cellular aging is oxidative stress caused by the level of pro-oxidants outweighing the level of antioxidants.

Aging is a process exemplified by several biological changes due to an increase in the production or activities of certain enzymes or physiological processes leading to the functional decline of cells over time (Ferrucci et al., 2020). It is a progressive and irreversible decline in physiological function leading to age-related diseases, like neurodegenerative diseases, musculoskeletal disorders and arthritis, cardiovascular diseases, cancer, and hormonal imbalance (Guo et al., 2022). These diseases can result in a heavy socioeconomic challenge to society (Lama, 2023). There is a global increase in the population of older people, and it is estimated to double from 12% in 2015 to 22% by the year 2050 (Mitchell and Walker, 2020). Although the prospect of an increase in lifespan is highly welcome, there is a need to accompany the longer lifespan with healthy and comfortable living rather than longer years with disability and disease (Robine, 2021). Therefore, it is necessary to improve the pathophysiological conditions associated with the process of aging by identifying effective therapeutics as anti-aging interventions, to mitigate the health burden associated with the increase in the population of older persons in societies (Du et al., 2021).

Fruits are well-known to possess different secondary metabolites with varying pharmacological properties. Polyphenols are phytoconstituents with free radical scavenging properties that could act as antioxidants which play a significant role in reducing oxidative stress associated with aging (Rudrapal et al., 2022). Other bioactive plant secondary metabolites like anthocyanins, catechins, and isoflavones have effective scavenging capacity against free radicals (Dhalaria et al., 2020). Frequent consumption of fruits like citrus could provide several health benefits including pausing the process of aging and alleviating several chronic health disorders associated with aging (Bayrami et al., 2020). The presence of high bioactive content in these fruits aids in delaying the processes of aging and alleviates the risk of various age-linked chronic diseases (Dhanjal et al., 2020).

C. sinensis has been reported to possess the ability to reduce inflammation in human keratinocytes, providing protection against ultra-violet skin damage, and antioxidant properties in human subjects (Nobile et al., 2022). However, the anti-aging properties of *C. sinensis* have not been exhaustively researched. This study aimed to evaluate the potential of *C. sinensis* juice in ameliorating some pathological conditions associated with aging using a D-galactose aging-induced rat model.

2.0 Materials and Methods

Citrus sinensis juice preparation

Citrus sinensis (sweet orange) fruits were obtained from a local market at Iyana-Iba, Ojo local government area of Lagos state, Nigeria. The fruit was authenticated at the Department of Botany, Lagos State University. The fruits were thoroughly washed, and the juice was extracted using a juice extractor. The fruit debris was removed by centrifugation at 1500 g for 5 min and then filtered using a muslin cloth. The filtrate was concentrated using a freeze-dryer.

2.1 Experimental animals

Twenty female Wistar rats of an average body mass of 200 g were distributed randomly to four different cages (five rats per cage) and housed in a well-ventilated animal house at the Lagos State University for a week to acclimatize. The animals were maintained at room temperature with a 12-hrs. day and 12-hrs. night cycle. They had unrestricted access to standard rat pellets and clean water. The procedures were performed following all instructions as stipulated by the Guide for the Care and Use of Laboratory Animals (Care and Animals, 1986) and approved by the Research Ethical Committee of the Lagos State University (LASU/23/REC/117).

2.2 Experimental design

The rats were randomly assigned into four different groups. The first group was named the control and was given distilled water. Aging was induced in animals in the other three groups by subcutaneously administering 150 mg/kg body weight of D-galactose. In addition, one of the aging-induced groups (named the model group) received distilled water while the remaining two groups were treated with orange (500 mg/kg bw) and quercetin (15 mg/kg bw), both suspended in water respectively. The extract and standard (quercetin) were administered orally using an orogastric tube for 28 days. After the treatments, the animals were sacrificed, their blood was collected, and the serum was immediately separated. The rats were dissected, and organs of interest (brain, heart, liver, and kidneys) were harvested. The organs were homogenized using 0.25 M ice-cold sucrose solution. The homogenates supernatant was recovered after centrifugation at 105 g × 10 min for the antioxidant assays.

2.3 Serum glucose concentration

Serum glucose concentration was assayed using the glucose oxidase method as reported by Trinder (Trinder, 1969).

Serum lipid profile

The Quimica Clinica Aplicada assay kits were used to measure the level of triacylglycerol (TG), Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), as described by Ashafa and Kazeem (Ashafa and Kazeem, 2015).

2.4 Inflammatory markers assay

Commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Shanghai Jingkang Bioengineering Co., Ltd) was used to assay the level of serum tumour necrosis factor-alpha (TNF-α), and interleukins (IL-6, and IL-10) following the manufacturer's instructions.

2.5 Hormonal assay

Reproductive hormones estradiol (EST), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were assayed using AccuBind Enzyme-Linked Immunosorbent Assay (ELISA) kits (Calbiotech Inc.) following the accompanying kit protocol for each parameter.

2.6 Tissue antioxidant enzymes' assay

Tissue concentration of catalase and superoxide dismutase were determined in the supernatants obtained from the centrifugation of brain, heart, liver, and kidney homogenates using standard assay kits.

2.7 Statistical analysis

GraphPad Prism 8 statistical package (GraphPad Software, San Diego MA, USA) was used to analyse all data obtained. Data were expressed as means ± SEM of five replicates and were compared using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Statistical significance was considered at $p < 0.05$.

3.0 Results

3.1 Effect of C. sinensis on blood glucose level in galactose-induced aging in rats

The effect of *C. sinensis* on blood glucose levels in galactose-induced aging in rats is presented in Figure 1. The glucose level in induced-aging animals increased when compared with the control group. Also, *C. sinensis* reduced the blood glucose in D-galactose-aging-induced animals compared to the non-treated group, however the difference was not significant ($p > 0.05$). The effect of the standard (quercetin) on the glucose level was comparable to C. sinensis.

3.2 Effect of C. sinensis on serum lipids

The effect of the administration of *C. sinensis* on lipids concentrations in aging rats is presented in Figure 2. D-galactose-induced aging increased triglyceride, cholesterol, and LDL-cholesterol in the rats (Figs. 2a, 2b, 2d). However, the concentration of HDL-cholesterol decreased in the aging rats (Figure 2c). The administration of *C. sinensis* significantly restored the concentrations of these lipids when compared with model-aging rats ($p < 0.05$).

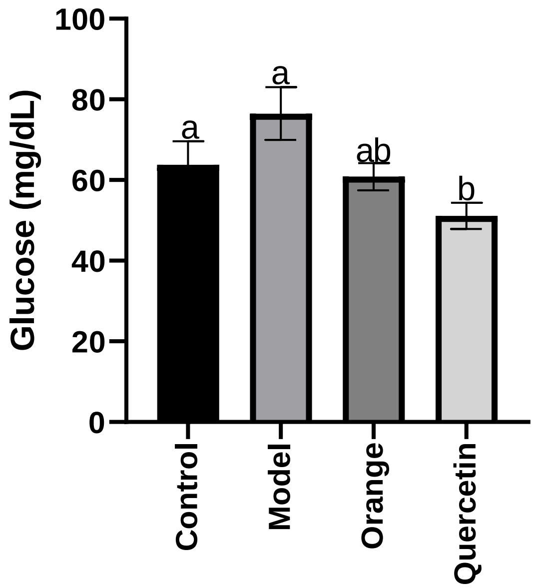

Figure 1: Effect of C. sinensis administration on blood glucose concentration in aging in rats. The mean values of groups with different superscripts are significantly different (P < 0.05).

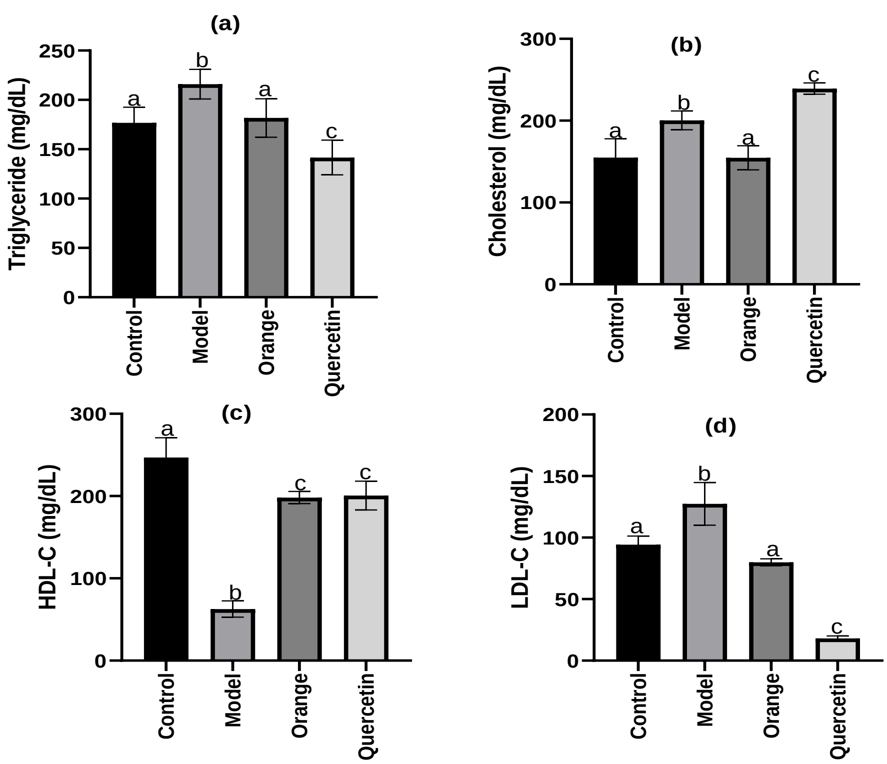

Figure 2: Effect of C. sinensis administration on the level of lipids in aging rats (a) triglyceride (b) cholesterol (c) HDL-cholesterol (d) LDL-cholesterol. Groups with different superscripts are significantly different (P < 0.05).

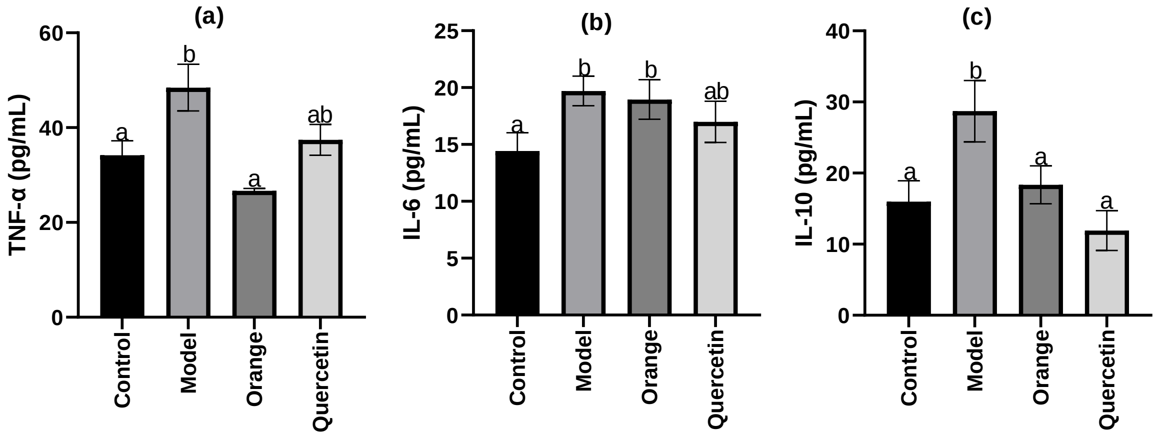

Figure 3: Effect of C. sinensis juice on inflammatory markers (a) tumour necrosis factor (TNF-α) (b) interleukin-6 (IL-6) and (c) interleukin-10 (IL-10) in aging rats. Groups with different superscripts are statistically different (P < 0.05).

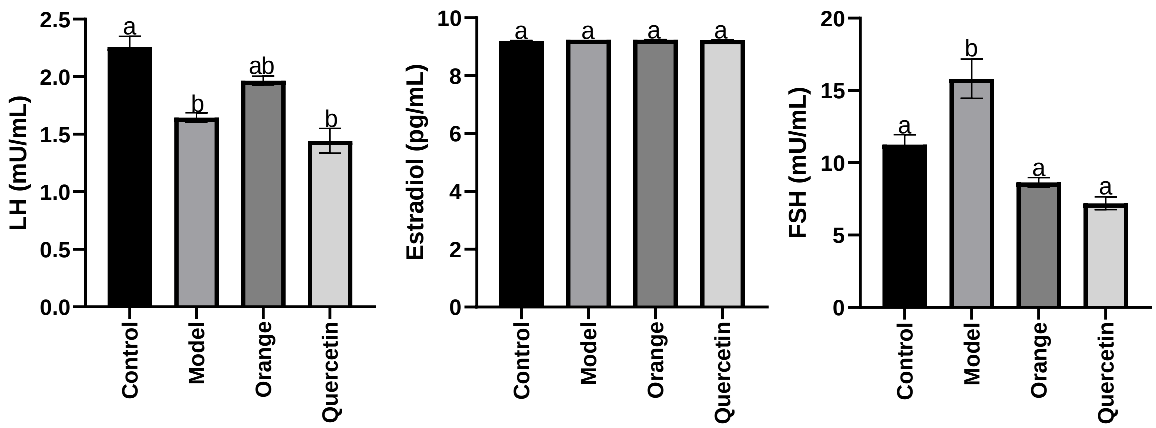

Figure 4: Level of female sex hormones in aging rats administered *C. sinensis* juice (a) luteinizing hormone (b) estradiol and (c) follicle-stimulating hormone. groups with different superscripts are significantly different (P < 0.05).

3.3 ***Anti-inflammatory activity of C. sinensis in aging rats***

The effect of *C. sinensis* juice on the level of inflammatory markers in the serum of aging rats is presented in Figure 3. The markers tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10) increased following induction of aging using D-galactose. Oral administration of the juice significantly reduced the level of TNF- α (Figure 3a), and IL-10 (Figure 3c) when compared with the non-treated animals. Although the *C. sinensis* extract also reduces the level of IL-6, the difference when compared with the model was not significant.

3.4 Effect of C. sinensis on sex hormones in aging rats

The level of female sex hormones in aging-induced rats treated with *C. sinensis* is shown in Figure 4. The result shows that a significant reduction in luteinizing hormone (LH) after D-galactose-induced aging was reversed in rats administered the *C. sinensis* juice extract (Fig. 4a). There was no statistical difference in the level of estradiol across the four groups (Fig. 4b). However, the increased level of follicle-stimulating hormone (FSH) in aging rats, was significantly reversed in group administered *C. sinensis* and the standard quercetin.

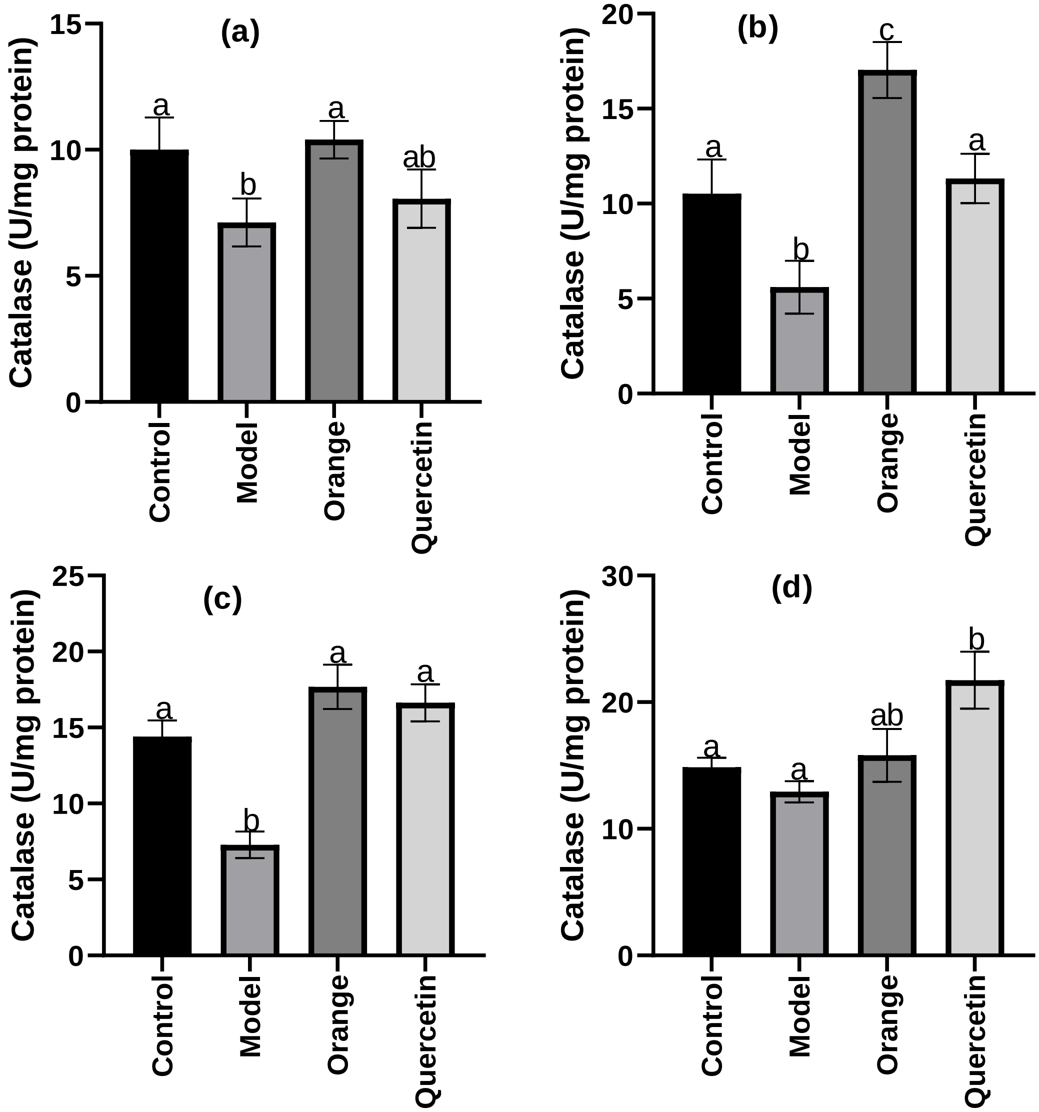

Figure 5: Catalase activity in tissues of aging rats administered *C. sinensis* juice in the (a) heart (b) liver (c) brain and (d) kidney. groups with different superscripts are significantly different (P < 0.05).

3.8 Tissue antioxidant enzymes activities in aging rats administered C. sinensis.

Catalase activity in the heart, liver, brain, and kidney in aging rats administered *C. sinensis* juice is presented in Figure 5. The result shows a significant reduction ($p < 0.05$) in the catalase activity in the heart, liver, and brain. However, the kidney catalase activity of the model group is similar to the control. There was a significant increase in catalase activity in the four tissues after the administration of *C. sinensis* ($p < 0.05$). Figure 6 shows superoxide dismutase (SOD) activities in the heart, liver, brain, and kidney of aging rats administered *C. sinensis* juice. Administration of D-galactose caused reduction SOD activity in the brain and liver of the aging rats (Fig. 6b and 6c). Administration of the C. sinensis juice significantly increased the SOD activity in the heart, liver, and brain (Figure 6a, 6b, and 6c) ($P < 0.05$), but did not influence kidney SOD activity.

4.0 Discussion

Aging is an inevitable process of life linked to several pathological conditions such as oxidative stress, dyslipidemia, inflammation, and hormonal imbalance which can affect many functionalities and the well-being of humans (Dharmarajan, 2021). In recent years, the search and discovery of anti-aging substances has become an emerging issue. Discovering anti-aging compounds in foods and natural products is desirable because of the general belief that they are safe and should be readily available (Hano and Tungmunnithum, 2020). This study assessed the anti-aging potential of *C. sinensis* (Sweet orange), by monitoring its ameliorating properties on some pathological

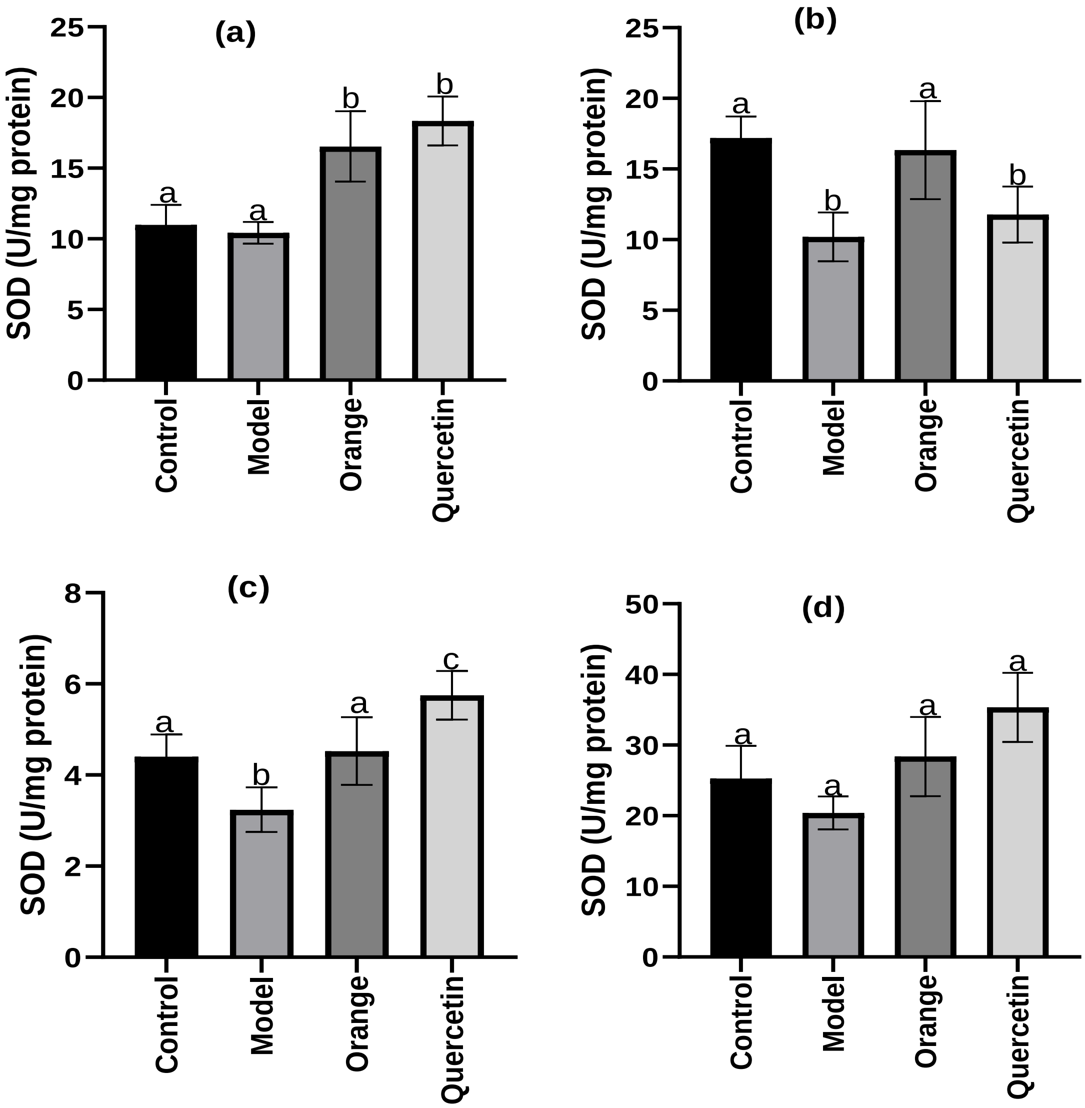

Figure 6: Superoxide dismutase activity (SOD) in tissues of aging rats administered *C. sinensis* juice (a) heart (b) liver (c) brain and (d) kidney. Groups with different superscripts are significantly different (P < 0.05).

conditions associated with aging. D-galactose-induced aging is an established model of an aging animal employed in the study of biochemical, morphological, and functional changes in animals associated with aging. The mode of action of D-galactose in inducing aging is reported to be oxidative stress and inflammation that may eventually result in severe damage to mitochondrial DNA resulting to apoptosis (Azman et al., 2021). Aging is linked with blood glucose level elevation which may emanate from a reduction in the sensitivity of insulin, resulting in more glucose-dependent insulin secretion through overexpression of calcium-sensing receptors (Salmon, 2016). In this study animals administered D-galactose for induction of aging has an elevated blood glucose level, however, there was an indication that the treatment with *C. sinensis* juice modulated the increased blood glucose. There are reports that oxidative stress which is rife in inducing aging could induce insulin resistance, and subsequently, hyperglycemia which may eventually result in diabetes (Omidkhoda et al., 2020). The reduction in elevated blood glucose levels by *C. sinensis* observed in this study agrees with an earlier report by Mallic and Khan, who postulated that the anti-hypoglycemic property of the juice may be due to the high presence of monoterpenes (Mallick and Khan, 2015).

Dyslipidemia is one of the metabolic diseases linked with high blood glucose levels and diabetes associated with aging (Consitt et al., 2019). Dyslipidemia is known to be one of the major causes of cardiovascular diseases which accounts for most deaths in about 25% of people aged between 64 and 85 years in the United States of America (Chia et al., 2018). Dyslipidemia is often interpreted as an increased concentration of total cholesterol, LDL-C, and triglyceride (TG) and a reduction in the level of HDL-C (KiliÇ et al., 2021). Results from this study show that *C. sinensis* juice could mitigate aging-associated dyslipidemia by reducing the level of LDL-cholesterol and increasing the level of HDL-cholesterol in aging rats. An increase in total cholesterol, plasma LDL, and a decrease in HDL-cholesterol are regarded as part of the normal aging process (Daskalova et al., 2021). Variation in lipid levels, resulting in an increase in the level of plasma triglyceride and reduction in HDL-C is an early sign of underlying metabolic syndrome. This condition in association with cardiovascular disease, leads to increased arterial stiffness in the aging population (KiliÇ et al., 2021).

Aging is a multifactorial process that is linked with several biochemical and molecular processes, however, the accumulation of reactive oxygen species (ROS) as a result of imbalance between the production and elimination of ROS causes tissue damage and immune dysfunction (Guo et al., 2020). Managing these pathological diseases associated with aging majorly focuses on bioactive compounds with antioxidant properties. Catalase and SOD activities were monitored in the heart, liver, brain, and kidney in galactose-induced-aging rats treated with *C. sinensis* juice and there was an indication that the extract could modulate the activities of these antioxidant enzymes. Reactive oxygen species (ROS) are generated from several chemical and metabolic reactions. These ROS generated induce oxidative stress by altering the balance between ROS and antioxidant defence leading to accelerated aging and its complications in cells and tissues (Li et al., 2018). A major tissues mechanism of combating oxidative stress is by production and the use of endogenous antioxidant enzymes like SOD, catalase, and glutathione peroxidase (GSH-Px) to maintain a balance between free radicals and antioxidants (Marwicka and Zięba, 2021). An increase in antioxidant enzyme activities in aging rats treated with the juice extract indicates that the juice could help reduce oxidative stress by increasing the enzyme activities to mop up the free radicals generated because of aging. The natural innate antioxidant system is regarded to be inadequate in solely preventing damages triggered by excessive free radicals in aging tissues (Hajam et al., 2022). Therefore, compounds with antioxidant properties could play a vital role by interacting with free radicals or enhancing the innate antioxidant enzymes to terminate the oxidative chain reactions (García-Sánchez et al., 2020). Also, natural products with antioxidant capacity are more beneficial than synthetic antioxidants because of their side effects (Li et al., 2018). The finding of this research on the juice enhancing SOD and CAT activity is in accordance with the report of Wang et al., who stated that treatment of *C. elegans* with orange extract significantly increases the activities of SOD and CAT (Wang et al., 2020).

Another biochemical process linked with aging is chronic inflammation (Chung et al., 2019). It has been reported that damage to cells because of excessive ROS stimulates the immune system causing the production, infiltration, and activation of immune cells like macrophages and other pro-inflammatory cytokines (Rendra et al., 2019). Elevated levels of interleukins and tumour necrosis factor-α (TNF-α) are generally observed in tissue aging (Grenier et al., 2021). Thus, assessing the anti-inflammatory potential of C. sinensis as a source of an anti-aging natural product is desirable. The anti-inflammatory activity of *C. sinensis* was assessed by evaluating the serum concentration of TNF-α, IL-6, and IL-10 in galactose-induced aging in animals. This study showed that the sweet orange juice significantly reduced the level of TNF-α and IL-10. Chronic inflammation has been observed in many aging cells and models. The alteration in the response of inflammatory markers in older organisms has been postulated to be a major contributor to tissue dysfunction in aging (Neves and Sousa-Victor, 2020). Studies have reported alteration in the levels of circulating pro-inflammatory and anti-inflammatory cytokines in some geriatric disorders such as sarcopenia and dementia. Elderly individuals experiencing sarcopenia exhibit a compromised “inflammatory status” which is characterized by higher proinflammatory markers IL-6, and TNF-α as well as anti-inflammatory cytokine IL-10 concentrations (Rong et al., 2018). The findings from this study suggest that C. sinensis juice possesses the ability to mitigate against aging-related chronic inflammation.

Several diseases that are linked with aging are also sensitive to changes in the immune system. It has also been reported that the aging process could be influenced by hormones which in turn affect the immune system (Gubbels Bupp et al., 2018). Hypothalamic-pituitary-gonadal (HPG) hormones including luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estrogen have multiple effects on the development, maintenance, and function of the brain, and the fluctuation in their levels have been implicated in some geriatric associated diseases such as psychiatric and neurological diseases (Gurvich et al., 2018). It has been reported that follicle-stimulating hormone (FSH), and Luteinizing hormone (LH) increased, while estradiol reduced in D-galactose-induced animal models (Li et al., 2019). However, while results from this study showed an increase of FSH in galactose-induced aging animals, there was no decrease in the level of estradiol, but the level of LH decreased. This suggests that aging may not necessarily affect the concentrations of these hormones, but instead affect the quality of the hormones. Also, it is a suggestion that the effect of aging on hormones could vary in individuals. Hormonal replacement is now commonly used to improve the quality of life in the aging population. Estrogens are used for the treatment of osteoporosis and to prevent vertebral and non-vertebral fractures (Son et al., 2019). However, even when there is no decrease in the production of these sex-hormones, receptor sensitivity could be decreased, which will result in impaired endocrine function (Sansone and Romanelli, 2021).

5.0 Conclusion

The irreversible pathophysiological process of aging which presents itself as a decline in tissue and cell functions resulting in increased risks of various aging-related diseases, such as oxidative stress, dyslipidemia, inflammation, and hormonal imbalance could be ameliorated using natural products. Oral administration of sweet orange juice for 28 days reduced the elevated blood glucose, and cholesterol in aging rats, as well as modulated antioxidant enzymes, inflammatory markers, and sex hormones in D-galactose-induced aging rats. Therefore, it can be concluded that daily consumption of *C. sinensis* fruit juice may alleviate the hyperglycemia, dyslipidemia, and inflammation experienced in aging Wistar rats.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors Contribution

Conception: HAB, MIK
Design: HAB, MIK
Execution: OA, KOY, AO
Interpretation: HAB, MIK
Writing: HAB

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