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Original Research

Effect of ethanol leaf extract of *Moringa oleifera* on D-galactose induced changes in lipid profile of aged wistar rats

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ABSTRACT

Uncontrolled hyperlipidaemia is linked to some age-related diseases like obesity, diabetes mellitus, and cardiovascular diseases. To examine the blood lipid profile in wistar rats, this study employed D-galactose-induced aging as a model. The lipid profiles were analysed using standard methods. Thirty (30) male albino rats were randomly assigned into five (5) groups of six (6) rats each and fed with basal diets. Group 1 and 5 which served as the young and adult controls respectively received basal diet only while the others were administered D-galactose (150mg/kg) and *Moringa oleifera* extract (MOLE-200mg/kg and 400mg/kg) respectively for eight (8) weeks. At the end of the study rats were sacrificed and blood samples were collected. At week 0, the results of the total cholesterol concentrations (98.81 ± 6.43), TAG (69.15 ± 1.34), LDL-cholesterol (31.80 ± 4.10), VLDL-cholesterol (13.83 ± 0..27) and HDL-cholesterol (35.64 ± 2.81) in rats administered *M. oleifera* leaf extract, D-galactose and distilled water respectively showed no significant ($P > 0.05$) difference across all the groups but at week 8 treatment with high dose of MOLE resulted in a significant ($P \leq 0.05$) decrease in total serum cholesterol (112.45 ± 3.05), TAG (114.05 ± 5.47), LDL-cholesterol (19.66 ± 4.06) concentrations relative to the D-galactose only treated group. Conversely, HDL-cholesterol concentration was significantly increased ($P \leq 0.05$) following treatment with both low (69.53 ± 1.94) and high dose (69.99 ± 2.64) of MOLE relative to the D-galactose (63.65 ± 2.54) treated group. These results suggest that *Moringa oleifera* leaf extract exerts an anti-hyperlipidaemic effect against D-galactose-induced toxicity and thus could confer protection against aging.

Keywords: Aging, D-galactose, lipid profile, *Moringa oleifera* leaf extract.

INTRODUCTION

Dyslipidaemia is a major cause of cardiovascular disease (Atkins and Wannamathee, 2020), which is a major cause of mortality in both developed and developing countries (Tsao *et al*., 2022). High blood cholesterol is a key risk factor for diseases; it caused 4.0 million deaths and 88.7 million disability-adjusted life years globally (GBD, 2015). Uncontrolled hyperlipidaemia and increased sedentary lifestyle of people, 20 years and above has led to increase in obesity. It is believed that there were over 2.3 billion children and adults are living with overweight and obesity and that if the current trends continue, 2.7 billion adults could be living with overweight or obesity by 2025(WHF, 2023).

Aging is a natural process that is complex and unstoppable (Argyropoulou *et al*., 2013). The use of D-galactose (D-gal)-induced aging animal models is a common practice to study the effects and mechanisms of natural compounds on oxidative stress damage (Liu *et al*., 2018). Bioactive agents from plants have been the focus of intensive research activities in recent times because of their pharmacological activity and have shown potentials in the management of many diseases. (Luetragoon *et al*., 2020). Numerous studies have been conducted to explore the possible effects of incorporating plant ingredients into safe medications through synthetic methodologies, as well as incorporating them into daily diets (Johnlouis *et al*., 2024; Onyeabo *et al*., 2021). Medicinal plants have been shown to possess ameliorative effects on diseases including reversing renal impairments (Ijeh and Ukweni, 2007), hepatic dysfunction (Ijeh and Obidoa, 2010), hyperlipidaemia (Ijeh *et al*., 2014) and hypertension (Ijeh and Ejike, 2011).

Moringa oleifera, also referred to as horse radish or drumstick in English, is rich medicinal plant whose roots, leaves, flowers, green pods, and seeds have been used for medicinal purposes and also has been known to serve as

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vegetable for food across Asia and West Africa, mostly in the south eastern part of Nigeria (Ijeh *et al*., 2021). *Moringa oleifera* has been established in previous studies as an antioxidant- rich plant and is popularly used in teas and in ethnomedicine.

MATERIALS AND METHODS

Moringa oleifera leaves were collected from National Root Crops Research Institute, Umudike and identified by Prof. G. C. Osuagwu at the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike.

EXPERIMENTAL ANIMALS

Thirty (30) male Wistar rats were used for the study. Six are four-weeks old (75-97g) and 24 are twenty- weeks old (250-390g). They were obtained from the Animal Care and Production unit of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The rats were acclimated for 7 days at the Animal House of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, with free access to feed (Chikun finisher) and water.

PREPARATION OF CRUDE EXTRACT / EXTRACTION

The pulverized sample (100g) was introduced into beaker and a known quantity of ethanol added to it (500mls), this was repeated 5 times. The mixture was stirred at intervals and allowed to stand at room temperature for 72 hours. The solution was filtered using a Whatman filter paper and the resulting filtrate was allowed to evaporate first with rotary evaporator and concentrated with oven set at 40^{o}C and then refrigerated. The percentage yield was calculated by the formula:

$$\% \text{ Yield} = \frac{weight\ of\ dried\ extract}{weight\ of\ sample\ used\ in\ extraction} \times 100$$

EXPERIMENTAL DESIGN

The rats were placed in 5 groups of 6 animals each, and the extract administered to the groups is as follows:

Group 1: Young animals administered distilled water only

Group 2: Aged animals treated with D-gal (150mg/kg) and low dose of *M. oleifera* (200mg/kg)

Group 3: Aged animals treated with D-gal (150mg/kg) and high dose of *M. oleifera* (400mg/kg)

Group 4: Aged animals treated with D-gal (150mg/kg) only

Group 5: Aged animals treated with distilled water only

A baseline study on lipid profile was carried out on the animals before commencing the administration of plant extract. The extracts were administered orally to the animals based on their body weights with the aid of an oral gavage. At week 0, blood samples were collected through the media cantus, centrifuged at 4000 rpm for 10 mins then the serum was decanted. At the end of the administration, the animals were sacrificed through cervical dislocation and blood samples collected via cardiac puncture for lipid profile analysis.

BIOCHEMICAL ANALYSES

Total cholesterol was determined using enzymatic colorimetric CHOD-pad test method described by Allain *et al* (1974) as reported in the Randox laboratory test kits.

Triacylglycerol (TAG) concentration in the serum was also determined spectrophotometrically using the method of Tietz (1990) explained in the Randox commercial kit. The high-density lipoprotein (HDL) concentration was determined by the precipitation method outlined by Grove (1979) while the serum low density lipoprotein (LDL) was calculated using the equation described by Friedewald *et al*. (1972).

STATISTICAL ANALYSIS

The data obtained were statistically analysed using one way analysis of variance (ANOVA) with the aid of Statistical Products and Service Solutions (SPSS) version 23. The means were separated using Duncan Multiple range test and results presented as mean ± standard deviation. The statistical significance was established at $P \leq 0.05$.

RESULTS

At week 0, the results of the total cholesterol concentrations (98.81 ± 6.43), TAG (69.15 ± 1.34), LDL-cholesterol (31.80 ± 4.10), VLDL-cholesterol (13.83 ± 0..27) and HDL-cholesterol (35.64 ± 2.81) in rats administered *M. oleifera* leaf extract, D-galactose and distilled water respectively showed no significant ($P > 0.05$) difference across all the groups but at week 8 treatment with high dose of MOLE resulted in a significant ($P < 0.05$) decrease in total serum cholesterol (112.45 ± 3.05), TAG(114.05 ± 5.47), LDL-cholesterol (19.66 ± 4.06) concentrations relative to the D-galactose only treated group. Treatment with the low dose showed a significant decrease ($P < 0.05$) in serum TAG (109.10 ± 11.98), VLDL-cholesterol (21.82 ± 2.40). Conversely, HDL-cholesterol concentration was significantly ($P < 0.05$) increased following treatment with both low (69.53 ± 1.94) and high dose (69.99 ± 2.64) of MOLE relative to the D-galactose (63.65 ± 2.54) treated group. (Table I, II, III, IV and V).

DISCUSSION

Elevated levels of cholesterol in the blood lead to atherosclerosis which may increase the risk of heart attack, stroke, and peripheral artery disease. The elevated cholesterol concentration as seen in D-galactose group suggests that D- galactose upregulates cholesterol absorption, through an interaction with SGLT1 which activates a protein kinase C pathway that regulates the activity of one of the intestinal cholesterol transporters that has been identified as hSR-BI (Play *et al* ., 2003) .

Table 1: Effect of *Moringa oleifera* on total cholesterol concentrations of experimental rats

TREATMENTS	WEEK 0	WEEK 8
GROUP 1	104.93±3.08[a]	108.93±3.52[a]
GROUP 2	98.81±6.43[a]	118.58±2.31[c]
GROUP 3	105.87±0.82[a]	112.45±3.05[ab]
GROUP 4	103.81±3.14[a]	122.17±4.12[c]
GROUP 5	103.71±5.03[a]	113.98±3.26[b]

Table II: Effect of *Moringa oleifera* on triacylglycerol concentrations of experimental rats

TREATMENTS	WEEK 0	WEEK 8
GROUP 1	69.68±2..21[a]	92.80±3.88[a]
GROUP 2	69.15±1.34[a]	109.10±11.98[bc]
GROUP 3	71.06±1.88[a]	114.05±5.47[c]
GROUP 4	69.77±2.33[a]	128.20±12.81[d]
GROUP 5	73.52±2.15[a]	102.13±6.42[ab]

TABLE III: Effect of Moringa oleifera on HDL-C concentrations of experimental rats

TREATMENT	WEEK 0	WEEK 8
GROUP 1	38.16±3.31[a]	68.67±2.25[b]
GROUP 2	35.64±2.81[a]	69.53±1.94[b]
GROUP 3	35.86±0.08[a]	69.99±2.64[b]
GROUP 4	36.30±2.97[a]	63.65±2.54[a]
GROUP 5	34.46±3.17[a]	68.57±2.49[b]

TABLE IV: Effect of Moringa oleifera on LDL-C concentrations of experimental rats

TREATMENT	WEEK 0	WEEK 8
GROUP 1	35.18±6.51[a]	21.70±4.85[ab]
GROUP 2	31.80±4.10[a]	27.23±3.77[bc]
GROUP 3	37.81±1.44[a]	19.66±4.06[a]
GROUP 4	35.74±5.89[a]	32.94±5.62[c]
GROUP 5	34.37±1.65[a]	24.99±5.75[ab]

The reduction in cholesterol could be attributed to the polyphenols in the *Moringa oleifera* which have been established to have strong antioxidant properties. Polyphenols inhibit lipid peroxidation by acting as chain breakers, peroxyl radical scavengers, and thus confer a protective effect on low density lipoprotein, preventing their oxidation. Polyphenolic compounds such as flavonoids decreases cholesterol by increasing their conversion to bile acids and elimination though faeces (Aborhyem *et al*., 2016). The flavonoids in *Moringa oleifera* leaves work by preventing LDL oxidation by donating H+ and inhibiting the activity of 3-hydroxy-3methylglutaryl coenzyme-A reductase (HMG-CoA Reductase) (Sari *et al*., 2022). This lowers elevated cholesterol levels, resulting in a substantial reduction in coronary events and deaths from disease conditions including Coronary Heart Disease (Zhu *et al*., 2019). *Moringa oleifera* also contains saponins whose anticholesterolaemic effects are well established. They bind with cholesterol in the intestinal lumen, preventing its absorption and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its faecal excretion (Liao *et al*., 2021). Another mechanism for the reduction could be attributed to interference with abnormal metabolic biosynthesis of cholesterol thus; normalization of the activity of hepatic HMG-CoA reductase, which is the first committed enzymatic step of cholesterol synthesis. This attribute could be associated to the flavonoid composition of *M. oleifera*. *Moringa oleifera* could also reduce the cholesterol concentration in the co-treated groups through inhibition of fat absorption. This could be attributed to tannin composition of *Moringa oleifera* since astringent tannins has been shown to react with mucosal proteins and small intestinal epithelial cells to inhibit fat absorption (Sari *et al*., 2022).

Low Density Lipoprotein (LDL) is known to associate with the development of atherosclerosis, and it is therefore widely studied as a potential risk factor of cardiovascular diseases (Ivanova *et al*., 2017).Increased serum triglyacylglycerols and very low density lipoprotein-cholesterol concentrations have been linked in the pathogenesis of disease conditions such as coronary heart disease and other cardiovascular conditions. Hence, LDL, VLDL and triglyceride concentrations are used as parameters for the prediction of cardiovascular diseases and also seen in diabetes mellitus.

Increase in the LDL, VLDL and TAG concentrations as seen in the D-galactose group could be indicative of cardiovascular disease. This agrees with the result of Xu *et al.* (2019) that D-galactose induced cardiovascular diseases including high blood pressure. However, their significant reduction following treatment with MOLE is an indicative of ameliorative effects of the fractions. The reduction in the LDL concentration could be as a result of inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor-mediated catabolism of LDL-cholesterol and increase in uptake of LDL from the blood (Maruthapan and Sakthi, 2010). Other mechanism of reduction could be attributed to phytochemical compositions of the extract. Niacin (B3), lowers LDL by selectively inhibiting hepatic diacylglycerol acyltransferase 2, reducing triglyceride synthesis and VLDL secretion through a receptor HM74 (Madsen *et al*., 2017) and HM74A or GPR109A

(Soudijn *et al*., 2007). Possibly the extract contained phytochemicals which are inhibitors of Protein convertase subtilisin/kexin type 9 (PCSK9). PCSK9 is secreted into the plasma where it binds to LDL receptors facilitating its lysosomal degradation. It reduces the expression of LDL receptors on the cell membrane thereby decreasing the clearance of LDL (Tsao *et al*., 2022).

HDL and its protein and lipid constituents help to inhibit oxidation, inflammation, activation of the endothelium, coagulation, and platelet aggregation. All these properties may contribute to the ability of HDL to protect from atherosclerosis. HDL transports cholesterol mostly to the liver or steroidogenic organs such as adrenals, ovary, and testes by both direct and indirect pathways. HDL function to remove cholesterol antheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL protect against cardiovascular disease (De Cabo and Mattson, 2019)

The significant reduction in the HDL concentration as seen in the D-galactose group could be indicative of platelet aggregation, oxidation or inflammation caused by the high concentration of D-galactose leading to the depletion of serum HDL. The significant improvement in the co-treated groups is indicative of hypolipodemic activity of the extract. This could be attributed to the phytochemical composition of the plant since hypolipidemic activity of plants has been linked to their saponin, flavonoid and polyphenolic compositions (Liao *et al.,* 2021). Higher HDL levels are correlated with lower risk of cardiovascular diseases (Deng *et al.,* 2022). Also the significantly lowered cholesterol level may have contributed to the observed significant high serum high-density lipoprotein cholesterol in the animals. About 30% of blood cholesterol is carried in the form of HDL. Significant lowering of total cholesterol and rise in HDL-C is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions (Eze *et al.,* 2012).

CONFLICT OF INTEREST

There was no conflict of interest.

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