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Original Research

Moringa seeds mitigate oxidative stress and promote antioxidant activity in aging male rats

Noha Sayed Hamed[*], **Hoda Badr Hammad, Mona Ibrahim Abdou**

ABSTRACT

Introduction: The aim of this study was to examine the antioxidant effects of one of the natural plant sources against oxidative stress caused by aging. *Moringa oleifera* seeds (MOS), the less utilized part, were chosen to investigate their role against oxidative stress in aging male albino rats. **Methods**: DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), polyphenols, some vitamins, and amino acids were estimated in the moringa seeds. The animals were mainly divided into two groups: adult and elderly rats. Each group was further subdivided equally into normal (not treated) and treated rats (which were orally administered an aqueous ground suspension of the MOS seeds) at a dose of 500 mg/kg body weight for four weeks (five days/week). Serum levels of free testosterone, free triiodothyronine (FT3), free thyroxine (FT4), and liver and kidney function were assessed. Additionally, histopathological investigations of brain and testicular tissue samples were conducted. Glutathione S-transferase (GST), malondialdehyde (MDA), and acetylcholinesterase (AChE) were measured in the homogenates of brain and testicular tissues. **Results**: The results reveal a powerful antioxidant effect of MOS, indicated by a significant reduction in MDA levels along with a significant increase in GST and AChE concentrations. MOS treatment significantly increased serum testosterone levels and thyroid hormone levels in the male rats' serum. **Conclusion**: MOS could improve most oxidative stress disorders associated with aging in male rats. Finally, the inadequacy of research on brain and testicular aging has been identified, and new research options have been proposed to aid in the treatment of brain and testicular aging. Further study in this area may uncover the underlying mechanisms, paving the way for the development of new treatments for age-related issues.

Key words: Moringa oleifera seed, aging, oxidative stress, brain, testicular tissues, testosterone

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INTRODUCTION

The aging process is defined as a gradual, unavoidable loss of several body functions[1]. As age progresses, the brain undergoes biological, psychosocial, neuroanatomical, and neurophysiological changes that are all associated with a decline in cognitive function[2]. There is accumulating evidence that increasing male age has a considerable detrimental influence on spermatogenesis and fertilization[3]. The spermatogenesis microenvironment reveals major anomalies with age, such as reduced number, organelle aging, aberrant hormone production, and deficiencies of the blood–testicular barrier[4]. Aging is characterized by a gradual decrease in tissue and organ function. According to the oxidative stress theory of aging, the accumulation of harmful reactive oxygen and nitrogen free radicals leads to age-related functional impairments. In addition, oxidative stress contributes to a number of age-related illnesses, such as sarcopenia and frailty (*e.g.*, cardiovascular diseases, obstructive pulmonary disease, diabetes, renal disease, neurodegenerative diseases, and cancer). Antioxidant defenses mitigate the detrimental effects of free radicals, which are produced by a range of internal and environmental processes. Oxidative stress is brought on by an imbalance between the production of free radicals and antioxidant defenses[5]. Phytochemical prevention of serious health conditions has gained global recognition, with studies on the anti-aging properties of plant extracts garnering substantial attention.

Moringa oleifera (MO) is a medicinal plant belonging to the family Moringaceae. The MOS has recently drawn a lot of interest because of its nutritional value and health advantages[6]. MOS (fresh, powdered, or cooked) includes a wide range of nutrients, such as significant sulfur amino acids, protein, and minerals[7]. Recently, bioactive peptides generated from natural plants have gained attention in the health and pharmaceutical industries[8]. According to Jain *et al.*[9], MOS contains around 52% of all necessary amino acids, making it a possible source of functional protein isolate. Because of the harmonized amino acid content, MOS protein could be utilized instead of

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other sources of protein for human diets. MOS protein contains significant levels of cysteine and methionine (43.6 g/kg protein), which are comparable to chicken eggs, cow, and human milk[10]. MOS has displayed a plethora of bioactivities such as antioxidant[11], anti-inflammatory[12], antimicrobial[13], antiviral[14], liver-protective[15], anti-diabetic[16], antitumor[17], and cardio-protective activities[18].

Previous research has concentrated on the utilization of MOS and its protective mechanisms in various tissues rather than the brain. As a result, the current study employs MOS to assess its effect as an antioxidant against oxidative stress produced by aging, such as serum biochemical disturbance and brain and testicular tissue damage.

METHODS

All materials were of analytical grade and were purchased from commercial stores.

Determination of DPPH

The free radical scavenging capacity of MOS was determined using the stable DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), according to Hwang and Do Thi[19].

Radical ABTS Scavenging Activity Determination

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) stock solutions were produced according to Hwang and Do Thi[19].

High-Performance Liquid Chromatography (HPLC)

The phytochemical, amino acids, and vitamin B composition of MOS were analyzed using HPLC. Fifty milligrams of MOS was combined with 5 mL of H_2O and 5 mL of HCl, heated at 100°C for 24 hours, and filtered. Finally, 1 mL of filtrate was dried, resuspended in 0.1 M HCl, and injected into the HPLC. HPLC analysis was performed using an Agilent 1260 series. The Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm) temperature was maintained at 40°C.

Preparation of MOS and Experimental Animal Groups

Dry Moringa oleifera seeds (MOS) were purchased from the laboratories of the National Research Centre, Giza, Egypt. MOS were washed and peeled. A mortar was used to grind and finely powder the seeds, which were then used as a suspension in distilled water. Twenty male Wistar albino rats were obtained from the animal house of the Nuclear Research Centre, Egyptian Atomic Energy Authority (EAEA). Before beginning the experiment, rats were kept in the Animal House of the Nuclear Research Centre's Radioisotopes Department for two weeks.

Rats were divided into four groups (five animals each): **G1**: five normal adult rats (2 months) without any treatment. **G2**: five normal adult rats (2 months) received orally 500 mg/Kg b.wt MOS (in a gradual increase of the dose from 100-500 mg/Kg b.wt during the first week) for 4 weeks (five days/week) according to El-Hak *et al.*[20], with some modification. **G3**: five aged (14 months) male rats without any treatment. **G4**: five aged (14 months) male rats received orally 500 mg/Kg b.wt MOS as in G2.

Sample Collection

Twenty-four hours after the last administration of MOS, we anesthetized the rats and humanely decapitated them to collect samples. Blood samples were collected without anticoagulants and centrifuged for five minutes at 3000 rpm to separate the serum. The rats' brain and testicular tissues from each group were subsequently collected.

Liver and Kidney Function

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzymes, albumin, urea, and serum creatinine were determined using assay kits obtained from Vitro Scient Co. (Cairo, Egypt).

Thyroid and Testosterone Determination by Radioimmunoassay

Serum FT3 (Cat. No. A13430), FT4 (Cat. No. 33880), and free testosterone (Cat. No. DSL4900) were determined using a commercial kit purchased from Beckman Coulter, Immunotech, Czech Republic.

Brain and Testicular Tissue Homogenate Preparation

The brain and testicular tissues of the rats in each group (10% wt/v) were promptly homogenized using a German IKA homogenizer. The tissue was cleansed with ice-cold PBS (0.01M, pH 7.4). Then, the homogenates were centrifuged at 5000 rpm for 15 minutes at 4°C with a Beckman Coulter Allegra 64R fixed-angle rotor (PN 392050).

Antioxidant Detection

Acetylcholine Esterase (AChE): AChE activity was measured according to Ellman *et al.*[21].

Glutathione S-transferase (GST) Kinetic Assays: GST activities towards 1-chloro-2,4-dinitrobenzene (CDNB) were measured spectrophotometrically at 340 nm according to Habig *et al.*[22].

Lipid Peroxidation: Lipid peroxidation (LPO) was assessed in terms of malondialdehyde (MDA) creation, according to the assay stated by Ohkawa *et al.*[23]. The MDA concentration was quantified as nmol MDA/mg protein.

Histopathological Examination

The brain and testicular tissue samples were collected in various groups and well-preserved in 10% formalin saline for one day. After washing with tap water, dehydration was accomplished using successive dilutions of alcohol (methyl, ethyl, and absolute ethyl). Specimens were cleaned in xylene and fixed in paraffin at 56°C in a hot air oven for one day according to Banchroft *et al.*[24].

Statistical Analysis

Results are represented as mean values ± standard error. A parametric test was used to determine whether the data had a normal distribution, and thereafter, various assessments were statistically analyzed using one-way ANOVA tests, followed by Tukey's HSD multiple comparisons as a post-hoc test, to identify the significant differences between the various groups. A p-value of < 0.05 was considered statistically significant. The software SPSS statistical version 20 (SPSS® Inc., USA) was used for all statistical evaluations.

RESULTS

DPPH and ABTS

The *in vitro* results indicated that DPPH scavenging activity had a value of 0.395 mg trolox/g, and the radical scavenging activities of ABTS were 0.802 mg trolox/g.

HPLC

The phytochemical, amino acids, and vitamin B of MOS were estimated using HPLC. The individual phenolic and flavonoid compounds results showed that chlorogenic acid and kaempferol had a strong response and more intense peaks (**Table 1** and **Figure 1**). The vitamin B content in MOS was estimated using HPLC. The results showed that vitamins B6 (2693.42 μg/g) and B12 (1577.69 μg/g) had the highest concentration, as shown in **Table 2** and **Figure 2**. The amino acid content in MOS is shown in **Table 3** and **Figure 3**.

In vivo results

Toxicity test of MOS

Acute oral administration of MOS up to 500 mg/kg resulted in no adverse effects in the experimental animals, including food intake, atypical body growth, decreased activity, diarrhea, bleeding, or mortality. As a result, no fatal dosage was established in this study.

Liver and kidney function

The results indicated that aging induced moderate changes in the biochemical parameters and an imbalance of oxidants and antioxidants in the male rats. There was a significant increase in serum urea, ALT, and AST levels in the aged rats compared to the control group. Treatment of rats with MOS significantly ($P < 0.05$) reduced urea levels in the serum of aged rats (**Table 4**). Furthermore, data showed that ALT, creatinine, and albumin levels were lower in MOS-treated rats than in control adult rats.

Effect of the MOS on testosterone and thyroid hormones

Aging showed a significant decrease in FT3, FT4, and free testosterone (**Table 4**). MOS treatment significantly increased serum testosterone levels and thyroid hormones in male rats.

Effect of the MOS on oxidative status

The results indicated that aging induced an imbalance of oxidants and antioxidants in male rats. Aging showed a significant increase in lipid peroxidation in testicular and brain tissues (**Table 5**). Treatment of rats with MOS significantly ($P < 0.05$) diminished the raised values of MDA in the aged group (**Table 5**). The abnormally declined activities of GST and AChE enzymes significantly increased in the old group administered MOS when compared with control. The administration of MOS dramatically increased GST and AChE levels compared to untreated rats.

Histopathological findings

The histopathological investigation of the testicular and brain tissue of different groups is provided in **Figure 4** and **Figure 5**, respectively. Table 6 illustrates the histopathological changes in the testes and brains of rats administered moringa or not, based on the scoring severity of injury.

DISCUSSION

The worldwide issue of population aging is becoming increasingly problematic as the global economy and medical care expand. Age-related disorders are

Table 1: Individual phenolic and flavonoid compounds of *moringa oleifera* seed (MOS)

MOS (1g/15 ml)			
Compounds	Area	Concentration (μg/ml)	Concentration (μg/g)
Gallic acid	30.83	2.43	36.46
Chlorogenic acid	28.42	4.30	64.52
Catechin	1.49	0.35	5.28
Coffeic acid	2.66	0.20	2.93
Syringic acid	2.17	0.19	2.78
Pyro catechol	16.38	2.38	35.69
Vanillin	41.11	1.42	21.32
Cinnamic acid	42.52	0.85	12.71
Kaempferol	18.02	2.82	42.36

Table 2: The vitamin B content in *Moringa oleifera* seed (MOS)

MOS (1g/15 ml)		
	Area	Concentration (μg/g)
Vitamin B1	11.65	437.14
Vitamin B2	5.37	76.50
Vitamin B6	35.15	2693.42
Vitamin B9	3.40	244.52
Vitamin B12	12.60	1577.69

affecting an increasing number of senior individuals[25]. Consequently, the demand for natural plant-based treatments that prevent and treat age-related illnesses is growing. This study employs MOS to assess its effects as an antioxidant and anti-aging agent against oxidative stress caused by aging, such as brain and testicular damage.

The phytochemical composition, vitamin B content, and amino acids of MOS used in this study are presented in **Tables 1 and 2** and **Table 3**. MOS has been shown to contain bioactive secondary metabolites such as flavonoids (catechin, kaempferol, vanillin), with the most abundant being kaempferol (42.36 μg/g), and phenolic acids (ellagic acid, gallic acid, ferulic acid, chlorogenic acid), with the most abundant being chlorogenic acid (64.52 μg/g). Manisha *et al.*[26] demonstrated that MO leaves contained flavonoids, mainly quercetin and kaempferol, with concentrations of up to 137.81 and 106.75 mg/g, respectively, and phenolic acids, which include ferulic acid, ellagic acid, and chlorogenic acid.

Infertility affects 50-80 million people worldwide, with males accounting for 20-50% of cases[27]. In the present study, the effect of MOS in enhancing male reproduction is clearly observed in the aged group compared to the control. The antioxidants in MOS worked in tandem with the epididymis's antioxidant system to further protect and improve the process of spermatogenesis. Aging may be associated with a rise in endogenous ROS, which reduces antioxidant enzyme activity, with Leydig cells being particularly vulnerable to this impact. High ROS levels can alter the hormonal balance that governs male reproductive processes by acting on the hypothalamic-pituitary-gonadotropic (HPG) axis[28]. Reduced LH secretion causes Leydig cells to generate insufficient testosterone[29], resulting in uncontrolled spermatogenesis and suppression of sexual behavior[30]. In spermatozoa, MDA molecules enter the cell membrane structure, disrupting the symmetric distribution of lipid membrane components. Lipid peroxidation destroys the central region of the sperm cell, resulting in a loss of acrosome ability for fertilization[31]. Moringa leaves might have beneficial effects on improving male sexual performance in stress-induced sexual dysfunction in rats[32]. Zade *et al.*[33] investigated the effects of

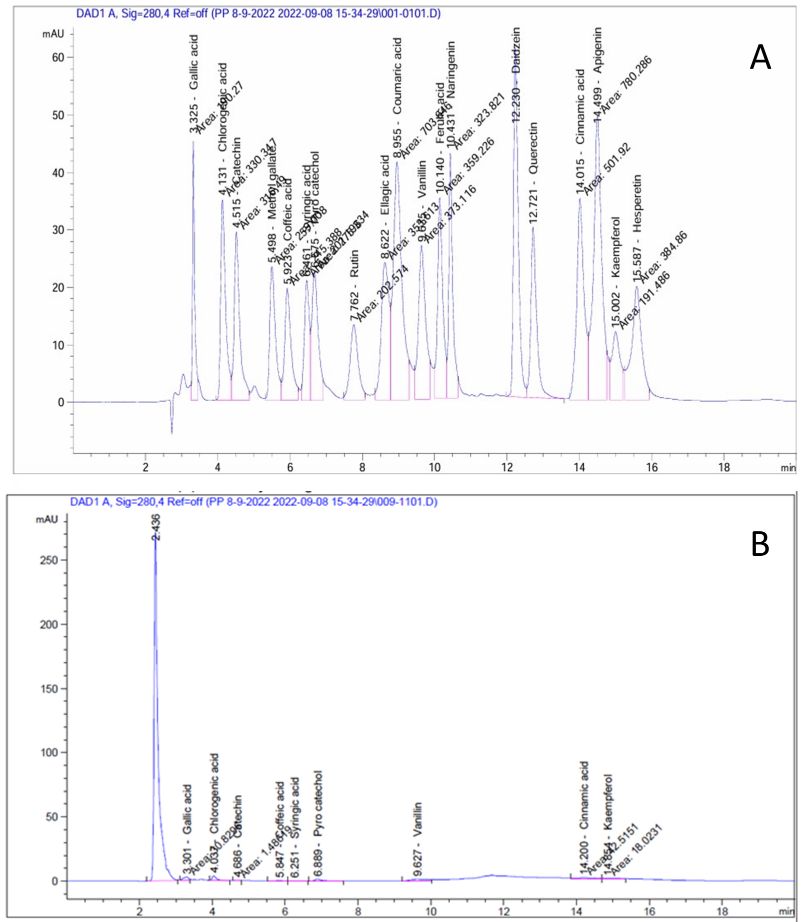

Figure 1: **Chromatographic profile (HPLC) of (A) polyphenolic standard compounds, (B) *Moringa oleifera* seed (MOS).**

the aqueous seed extract on the frequency of mounting and intromission in male rats. The investigators concluded that MOS may improve male sexual behavior because it also significantly boosts desire and sperm count. MO has been used to treat male sexual functions such as desire, erectile dysfunction, and testicular damage[34]. After oral administration of MO, aged rats (18-19 months old) showed improvements in sperm count and morphology, indicating its potential utility in the treatment of sperm abnormalities[35]. Furthermore, MO is a preventative approach for a variety of ailments and disorders, such as testosterone-induced benign prostatic hyperplasia[36].

This study provides findings on the role of MOS on brain cholinergic enzyme (AChE) and MDA. The G2 and G4 groups (MOS administration) presented a significant drop in MDA level and an increase in GST and AChE activities when compared to control groups. MOS contains high phenol and flavonoid content, which are crucial oxidative components in plants. Their antioxidant activity is attributed to their redox characteristics, which help scavenge and neutralize free radicals. MO is utilized to treat neurodegenerative illnesses, including Alzheimer's, ischemic

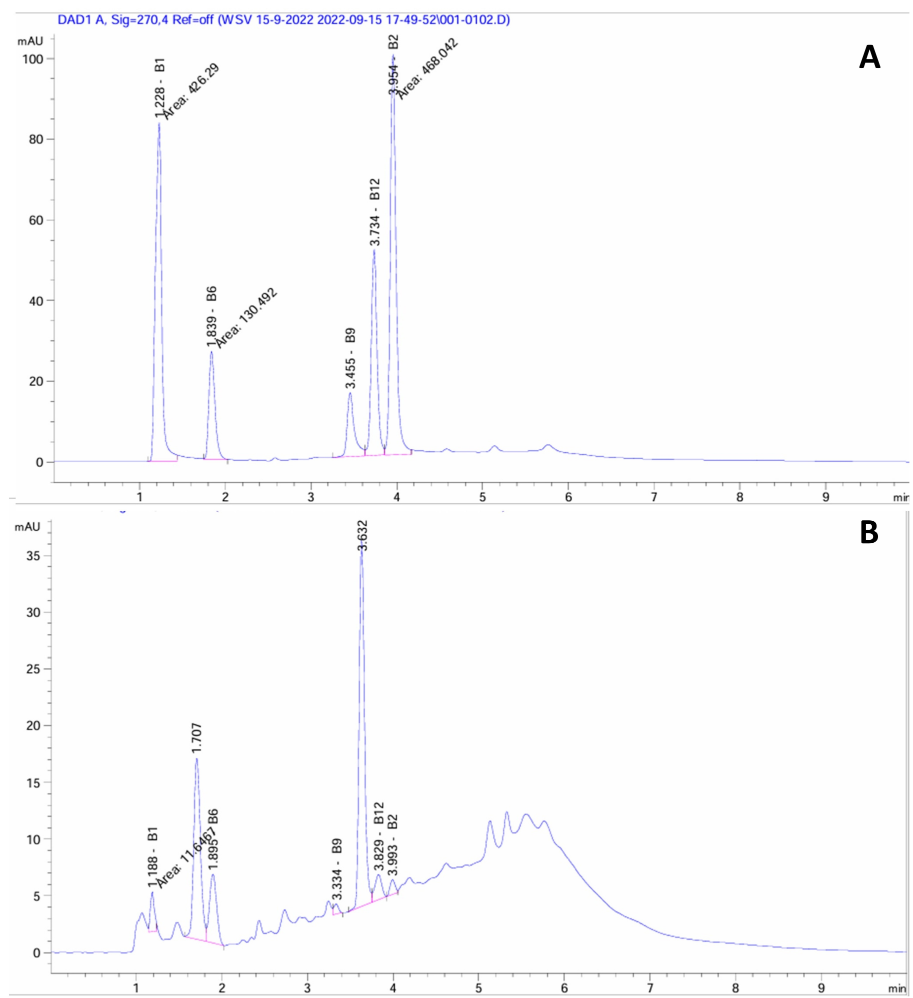

Figure 2: **Chromatographic profile (HPLC) of (A) vitamin B standard compounds, (B) vitamin B content in *moringa oleifera* seed (MOS).**

stroke, and epilepsy[37]. Aging of the brain can cause memory and cognitive impairment, which is commonly associated with changes in the structural flexibility of dendritic spines. In elderly animals and humans, spine number and maturity are reduced, along with alterations in synaptic transmission, which may represent abnormal neuronal plasticity intimately related to reduced brain function. In the most severe cases, a neurodegenerative illness that entirely destroys the basic processes of brain development is possible[38].

This study concluded that MOS improves the role of the thyroid by raising thyroid hormones (FT3 and FT4) and reducing oxidative stress in aged male rats when compared to adults. Tahiliani and Kar[39] revealed that MO leaf extract has therapeutic efficacy in managing hyperthyroidism, an autoimmune condition, by suppressing T3 production and release.

Our results showed that MOS treatment led to improvements in AST and ALT levels, suggesting a hepatoprotective effect. A modest dose of M. seeds (500 mg/kg B.W.) was more effective at lowering ALT, although larger doses (1000 & 2000 mg/kg B.W.) produced the lowest level when compared to the control group[20].

This study concluded that MOS treatment led to improvements in urea and creatinine levels, suggesting a renal protective effect. MO reduces inflammation and

Table 3: The amino acid content in *moringa oleifera* seed (MOS)

Compound	Area (Sample)	Concentration S (ng/g)	Concentration S (mg/g)
ASP	1426.98	10711189.79	10.71
GLU	6742.52	60270978.95	60.27
Serine	1632.96	8117751.79	8.12
Histidine	441.34	5915776.01	5.92
Glycine	3518.41	12872315.78	12.87
Threonine	1051.08	5883521.42	5.88
Arginine	5301.38	38192078.24	38.19
Alanine	2513.66	10227841.74	10.23
Tyrosine	461.20	3846887.14	3.85
Cystine	4.24064	597574.11	0.60
Valine	1817.97	9686963.72	9.69
Methionine	1072.21	5130091.14	5.13
Phenylalanine	1399.57	11036623.03	11.04
Isoleucine	1719.54	8218485.13	8.22
Leucine	2687.61	14500137.30	14.50
Lysine	221.63	3599818.29	3.60
Proline	454.56	10849480.01	10.85

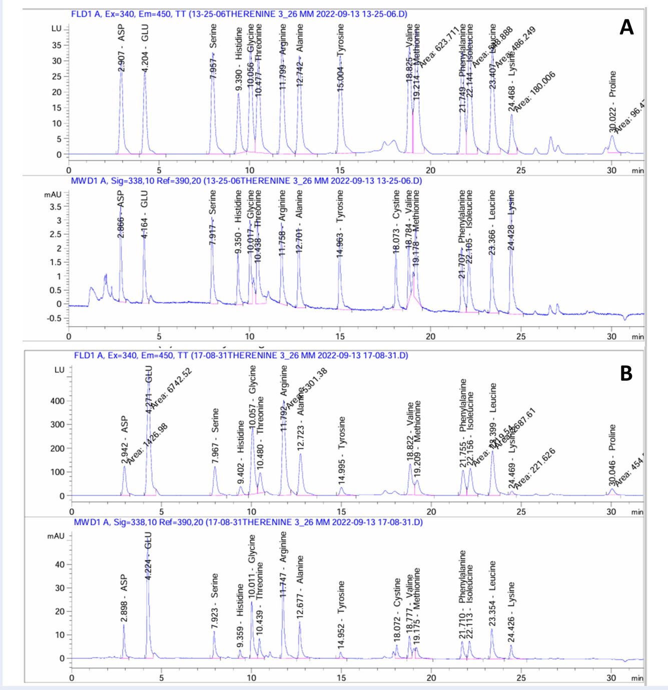

Figure 3: Chromatographic profile (HPLC) of (A) amino acids standard compounds, (B) amino acids content in *Moringa oleifera* seed (MOS).

Table 4: ($\overline{X} \pm$ SE) and Tukey test of serum liver functions, kidney function, thyroid hormones and free testosterone

Parameters	Groups			
	G1: Adult control (N = 5)	G2: Adult+MOS (N = 5)	G3: Old control (N = 5)	G4: Old+MOS (N = 5)
AST (U/L)	75.6 ± 1.02	72.8 ± 1.01	87.8 ± 2.8	78.2± 8 . 3
Tukey test	a	a	a	a
ALT (U/L)	25.2 ± 2.05	20.0 ± 0.54	40.4 ± 5.19	34.0±2.5
Tukey test	ab	a	c	bc
Albumin (g/dL)	3.0 ± 0.25	3.86 ± 0.13	2.54 ± 0.17	3.68±0. 20
Tukey test	ab	c	a	bc
Total protein (g/dL)	5.98 ± 0.18	6.50±0.266	4.72±0.33	6.90±0.25
Tukey test			a	
Urea (mg/dL)	54.8 ± 2.13	46.5±1.6	60.2±1.8	48.96±1.92
Tukey test	bc	a	c	ab
Creatinine (mg/dL)	0.46 ± 0.02	0.34±0.02	0.74±0.05	0.50±0.044
Tukey test	ab	a	c	
Free T3 (pg/mL)	5.62 ± 0.36	8.07±0.7	3.66±0.1	6.38±0.47
Tukey test		c	a	bc
Free T4 (ng/dl)	2.10 ± 0.02	3.6±0.31	2.0±0.03	3.1±0.2
Tukey test	a		ab	
Free testosterone (pg/ml)	14.3 ± 0.6	19.6±1.2	13.2±0.6	18.7±0.7
Tukey test		a	a	ab

Data expressed as mean ± SE, n = 5. Values with the same superscript in the raw are not statistically different. The groups are statistically significant ($P < 0.05$) as compared with control; using one-way ANOVA followed by Tukey's HSD multiple comparisons as a post-hoc test.

oxidative stress, which are common causes of kidney disease[40].

CONCLUSIONS

From the current study, it is clear that MOS can increase sexual behavior in male rats and could potentially have an impact depending on particular circumstances. MOS exhibited a significant decrease in the MDA level and an increase in GST and AChE activities in brain tissue. Additionally, MOS improves the role of the thyroid by raising the thyroid hormones FT3 and FT4. These findings highlight the crucial role of wild MOS bioactive components in brain, thyroid, and testicular function. Further study in this area may uncover the underlying mechanisms, paving the way for the development of new treatments for age-related issues.

ABBREVIATIONS

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), **AChE**: Acetylcholinesterase, **ALT**: Alanine aminotransferase, **AST**: Aspartate aminotransferase, **DPPH**: 2,2-diphenyl-1-picryl-hydrazyl-hydrate, **EAEA**: Egyptian Atomic Energy Authority, **FT3**: Free triiodothyronine, **FT4**: Free thyroxine, **GST**: Glutathione S-transferase, **HPG Axis**: Hypothalamic-pituitary-gonadotropic axis, **HPLC**: High-Performance Liquid Chromatography, **LH**: Luteinizing hormone, **LPO**: Lipid peroxidation, **MDA**: Malondialdehyde, **MO**: Moringa oleifera, **MOS**: Moringa oleifera seeds, **ROS**: Reactive oxygen species

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Table 5: ($\overline{X} \pm$ SE) and Tukey test of tissues antioxidant enzymes and lipid peroxidation

Oxidative marker Groups	Testicular GST (nmol/min/mg protein)	Testicular AChE (μmol/min/μg protein)	Testicular MDA (nmol/mg protein)	Brain GST (nmol/min/mg protein)	Brain AChE (μmol/min/μg protein)	Brain MDA (nmol/mg protein)
G1: Adult control	176.4 ± 2.83	4.98 ± 0.31	158.6 ± 2.2	235 ± 8.94	5.92 ± 0.24	149.4 ± 1.63
Tukey test						
G2: Adult+MOS	251.2 ± 5.49	9.24 ± 0.12	147 ± 1.87	336 ± 9.28	10.82 ± 0.32	134 ± 1.78
Tukey test	d	d	a	c	d	a
G3: Old control	97.0 ± 4.63	2.96 ± 0.166	209.4 ± 2.8	100 ± 20.7	4.12 ± 0.21	187.4 ± 1.63
Tukey test	a	a	d	a	a	d
G4: Old+MOS	215.8 ± 3.89	6.84 ± 0.172	183.8 ± 2.3	288.2 ± 4.8	7.88 ± 0.32	170.2 ± 2.57
Tukey test	c	c	c	c	c	c

Data expressed as mean± SE, n = 5. Values with the same superscript in the column are not statistically different. The groups are statistically significant ($P < 0.05$) as compared with control; using one-way ANOVA
followed by Tukey's HSD multiple comparisons as a post-hoc test.

Table 6: Histopathological alterations severity in the testes and brain

Organ	Histopathological alterations	G1: Adult control	G2: Adult+MOS	G3: Old control	G4: Old+MOS
Testes	Degenerated seminiferous tubules	+	-	+++	-
Brain	nuclear pyknosis and neuronal degeneration	+	-	+	-

+++ sever, ++ moderate, + mild, – nill

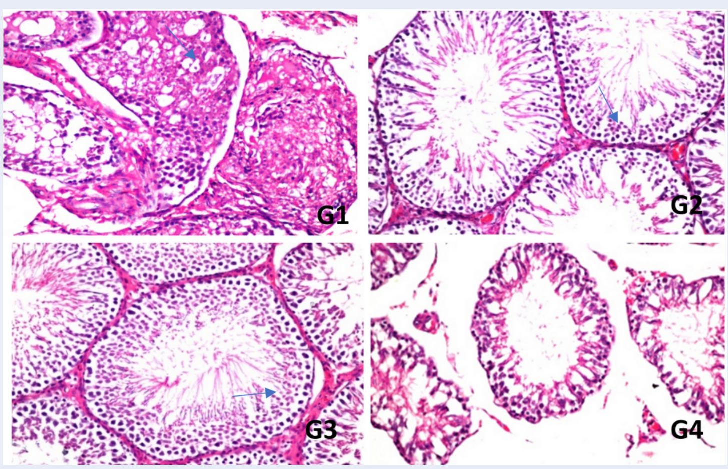

Figure 4: **Histopathological findings in testicular tissue. G1**: Adult rat testes showed degenerated seminiferous tubules with clotting of luminal spermatogonial contents. **G2**: old rats testes showed Shrinking and atrophy with irregular outline seminiferous tubules with lose of spermatogenic series. **G3**: Adult rats administered MOS showed normal histological structure of seminiferous tubules with couplet spermatogenic series in the lumen. **G4**: old rat administered MOS showed no histopathological alteration.

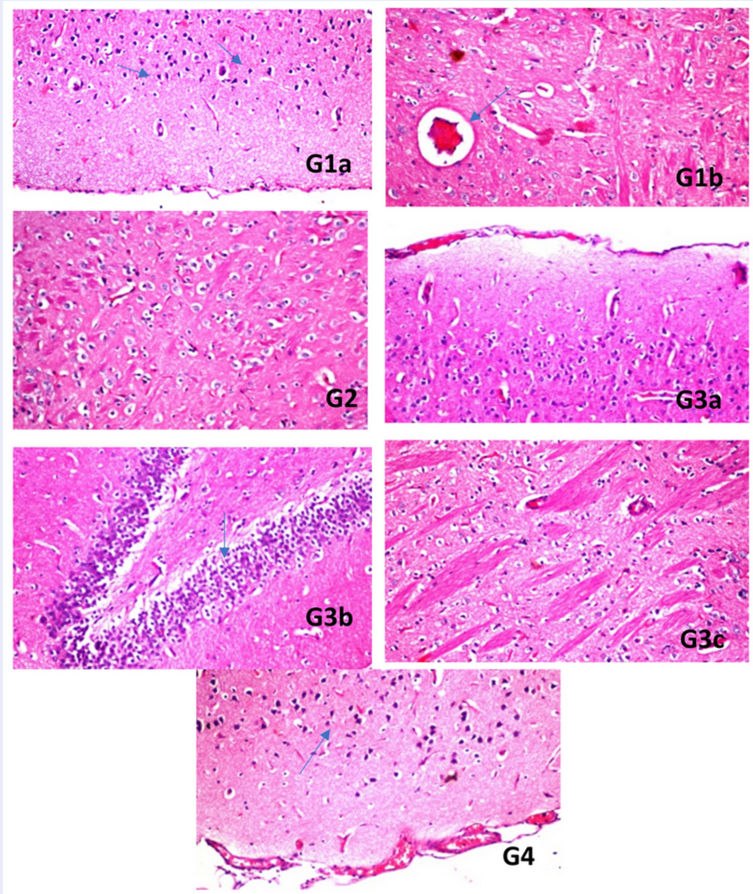

Figure 5: **Histopathological findings in brain**. **G1a**: cerebral cortex showing nuclear pyknosis and degeneration of the neurons. **G1b**: striatum showing congestion in blood vessels. **G2**: striutum showing intra cellular oedema of the neurons. **G3a**: cerebral cortex showing nuclear pyknosis and degeneration were noticed in some neurons. **G3b**: fascia dentate and hilus showing nuclear pyknosis and degeneration were observed in some neurons. **G3c**: striatum showing intracellular oedema in the neurons. **G4**: Cerebral cortex, congestion was observed in the meningeal blood vessels associated with nuclear pyknosis and degeneration of the neurons.

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AUTHOR'S CONTRIBUTIONS

Noha Sayed Hamed: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, conducted the experiments, Conceptualization. Hoda Badr Hammad: Editing, Validation, Data curation. Mona Ibrahim Abdou: Writing – review & editing, Formal analysis, Data curation, Conceptualization. All authors read and approved the final manuscript.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL

Ethical approval was sought and obtained from the Ethical Committee at the National Center for Radiation Research and Technology, EAEA (NCRRT-EAEA-12A/20).

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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