Original Research Article

EVALUATION OF THE THERAPEUTIC EFFECTS OF *MORINGA OLEIFERA* SEED OIL ON CADMIUM AND HERBAL ALCOHOLIC BEVERAGE – INDUCED PREFRONTAL CORTEX DAMAGE IN WISTAR RATS

ABSTRACT

The use of *Moringa oleifera* seed oil in the prevention of neurodegenerative diseases is on an increasing trend. Cadmium is one of the most toxic environmental pollutants causing many known damage to the brain, the consumption of herbal alcoholic beverages is known to cause neurodegeneration. The aim of this study was to investigate the ameliorative effects of Moringa oleifera seed oil on cadmium and herbal alcoholic beverage induced- damage to the frontal cortex of Wistar rats. Eighty Wistar rats were divided into eight groups of 10 rats each. Group A served as a control which received 2.5mg/kgbw phosphate buffer intra-peritoneally, while group F served as *Moringa*-treated control and received oral administration of 2.0 mg/kgbw*Moringa oleifera* oil. Groups B_1, B_2, D and E were injected intra-peritoneally with 3.5mg/kgbw $CdSO_4.8H_2O$ single dose. Group C_1, C_2 and D received oral administration of 0.5 ml Herbal Alcoholic Beverage (HAB) and group B_2, C2and E were administered orally with 2.0mg/kgbw*Moringa oleifera* oil for four weeks followed by sacrificed. Quantitative enzymes and biochemical antioxidant markers showed that cadmium and HAB administration caused a significant increase in AchE, SDH, CAT, GPx and MDA levels and decrease in SOD level. Conversely, there were significant decrease in AchE, SDH, CAT, GPx and MDA and increased

SOD level upon administration of *Moringa oleifera* oil. *Moringa oleifera* seed oil has natural antioxidant constituents that ameliorated damage caused by Cd and HAB.

Key Words: Cadmium, herbal alcoholic beverages, *Moringa oleifera* seed, frontal cortex

Introduction

"*Moringa oleifera Lam* (*Moringaceae*) is a highly valued plant distributed in many countries of the tropics and subtropics. It is the most widely cultivated species of the genus Moringa, which is the only genus in the family Moringaceae" [1]. "English common names include Moringa, benzolive tree and West Indian Ben. It is also known as drumstick tree, from the appearance of the long, slender triangular seed pods, horseradish tree, from the taste of the roots which resembles horseradish or Ben oil tree" [1]. "Moringa seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads, for fine machine lubrication and in the manufacture of perfume and hair care products" [2]. "It is an exceptionally nutritious vegetable tree with a variety of potential uses" [3]. "This tree has in recent times been advocated as an outstanding indigenous source of highly digestible Protein, Ca, Fe, Vitamin C and carotenoids suitable for utilization in many of the so-called developing regions of the world where undernourishment is a major concern" [4]. "Moringa leaves contain more vitamin A than carrots, more calcium that milk, more iron that spinach, more vitamin C than oranges and more potassium that bananas and that the protein quality of Moringa leaves rivals that of milk and eggs" [5]. In addition to its compelling water purifying powers and high nutritional value, *Moringa oleifera* is very important for its medicinal value.

“Various parts of this plant such as the leaves, roots, bark, flower, seed, immature pods and fruit act as cardiac and circulatory stimulants possess antitumor, antipyretic, antiepileptic, anti-inflammation, antifungal diuretic, cholesterol lowering antioxidant, anti-diabetic, hepatoprotective, antibacterial and are being employed for the treatment of different ailments in the indigenous system” [6].

“Cadmium (Cd) is a toxic metal in the environment, found in the soil, rock phosphate fertilizer and in tobacco plant. Cadmium is a highly accumulative toxicant with very long biological half-life” [7]. “It is not biodegradable and its levels in the environment are increasing due to industrial activities and human exposures to cadmium are inevitable” [7, 8]. “Acute Cadmium exposure produced toxicities in the lung, liver, testes and brain, while chronic exposure to Cd often leads to renal dysfunction, anemia, osteoporosis and bone fractures. Cadmium is a potent carcinogen in a number of tissues of rodents and classified as a human carcinogen” [9, 10]. “The neurotoxic effects of Cd have been reported in neonatal mouse brain[11] and young rat brain” [12].

“Cadmium produces oxidative damage to isolated rat optic nerve [13] and culture rat cortical neurons” [14]. “In humans, occupational exposure to Cd is associated with nerve psychological disorders [15] and Parkinsonism has been reported in a 64-year-old man exposed to Cd at a high dose” [16]. “Thus, accumulating evidence clearly indicates that Cd is neurotoxic in a number of settings. The mechanisms involved in neurotoxicity of Cd are poorly understood. Oxidative stress has been proposed as a mechanism for Cd toxicity in a number of tissues such as the kidney [17, 18] and brain” [19]. “Due to its low permissible exposure limit (0.5 g/L to 1.0 g/L) in human, over exposure may occur even in situations where trace quantities of cadmium are found. Exposure to cadmium is addressed in specific standards for the general industry, shipyard employment, construction industry and the agricultural industry” [20].

"Herbal alcoholic beverages commonly called *Ogogoro, alomo bitter, Opaeyin* in Nigeria. It is locally manufactured and packaged; it is consumed locally by the general public for sexual enhancement and as stimulants. Various investigations have revealed the deleterious effects of high percentage of alcohol (ranging from 17% to 70%) in most of the herbal alcoholic beverages" [21]. "Excess consumption of alcoholic beverages has been associated with high libido causing an excess sexual enhancement and over stimulation has also been linked to excess herbal alcoholic consumption. The overall effects of herbal alcoholic consumption have been revealed to cause health hazard leading to soft tissue damage such as cardiovascular, lung, liver, kidney and brain" [21].

"Frontal Cortex is the anterior part of the cerebrum, which extends from the superior frontal sulcus to the lateral frontal sulcus of (Sylvius) and aligned to the central frontal sulcus (of Ronaldo). Frontal Cortex plays a vital role in learning, personality and in information processing" [21].

The aim of this study was to investigate the ameliorative effects of *Moringa oleifera* seed oil on cadmium and herbal alcoholic beverage induced- damage to the frontal cortex of Wistar rats.

Materials and Methods

Eighty (80) Wistar Rats of both sexes were used for the research, *Moringa oleifera* seeds were procured from Ladoke Akintola University Farm in Ogbomoso, Nigeria and the plant specimen was identified with voucher number (No. FHI. 110266) assigned at Forestry Research Institute of Nigeria (FRIN), Jericho hill, Ibadan, where it was deposited.

Herbal Alcoholic Beverage (Local Market in Nigeria), Cadmium Sulphate (Guangzhou linhuada Chemical Reagent Co. Ltd., Guangdong, China)

The animals were divided into the following groups control group (A) received 2.5 mg/kgbw of phosphate buffer intraperitoneally single dose and the induced control groups (B_1 and B_2) received 3.5 mg/kgbw of $3CdSO_4.8H_2O$ [22]. Intraperitoneally and left for 72 hours. B_1 rats were maintained under normal laboratory condition for a period of four weeks and B_2 rats received 2.0 mg/kgbw of *Moringa oleifera* oil extract single dose daily for the period of four weeks. C_1 rats received 0.5 ml, 40% Herbal-gin via gavage, single dose daily four the period of four weeks while C_2 rats received 0.5 ml, 40% Herbal-gin and 2.0 mg/kgbw of *Moringa oleifera* oil extract simultaneously via gavage, single dose daily for the period of for weeks. Group D rats were injected intraperitoneally with 3.5 mg/kgbw of Cadmium sulphate ($3CdSO_4.8H_2O$) single dose and maintained for 72hrs [22]. Following oral administration of 0.5 ml, 40% Herbal-gin, single dose daily for the period of four weeks. The group E animal were also injected intraperitoneally with 3.5 mg/kgbw of Cadmium sulphate ($3CdSO_4.8H_2O$) single dose and maintained for 72hrs [22]. Following oral administration of 0.5 ml, 40% Herbal-gin and 2.0 mg/kgbw *Moringa oleifera* seed oil extract single dose daily for the period of four weeks. Group F rats received 2.0 mg/kgbw of *Moringa oleifera* seed oil via gavage, single dose per day for the period of four (4) weeks [22]. After the treatment, the animals were sacrificed via cervical dislocation.

Biochemical Analysis

The rats were sacrificed and the brain from all the rats were carefully excised from the skull using brain forcep, weighed and the frontal cortex from all the rats were homogenized in 5% sucrose solution at about 4^{o}C for quantitative enzyme assay for Succinate Dehydrogenase (SDH) [4] and Acetyl cholinesterase (AchE) [38] and oxidative stress markers Catalase **(**CAT**)** [39], Superoxide Dismutase **(**SOD**)** [40], Malondialdehyde **(**MDA**)** [41] and Glutathione Peroxidase (GPx) [4].

Statistical analysis

The data collected was subjected to statistical analysis, One- Way ANOVA was used with the computer software package model of graph pad prism version 5 and multiple comparism test was carried out and significant difference was observed and tested at $P < 0.05$, at percentage confidence interval of 95 (95% CI).

Results and discussions

Table 1 Effect of MO seed oil on the activities of Ache & SDH in Rats

Groups	A	B	B2	C	C2	D	E	F
AChE (10^{-5} moles/min/gm)	2.87±0.04	0.56±0.03*	4.63±0.95	0.54±0.02*	8.12±3.77	4.57±0.25	11.46±0.26*	13.50±0.52*
SDH (10^{-3} µ moles/min.mg)	4.13±0.04	5.41±0.199	5.39±0.08	1.81±0.01*	1.50±0.01*	8.11±0.03*	5.65±0.06	1.99±0.09*

Data are expressed as mean ± SEM

* It indicates significant level at $P < 0.05$ when compared with each other (multiple comparison test) and with the control group A.

Legend: AChE: Acetylchlinesterase, SDH: Succinate Dehydrogenase**, A:** Control, $\mathbf{B_1}$: Cadmium only, $\mathbf{B_2}$: Cadmium and Moringa oil, $\mathbf{C_1}$: HAB only, $\mathbf{C_2}$: HAB and Moringa oil, **D**: Cd and HAB, **E**: Cd + HAB + MO, **F**: Moringa oleifera seed oil

In table 1, an increase in the level of SDH activity was observed in groups B_1 (5.41 ± 0.19), B_2 (5.39 ± 0.07), D (8.11 ± 0.08) and E (5.65 ± 0.05) μmoles/min/mg respectively and this increase (P- value = 0.0001), was significant when compared with each other and with the control group A at $P < 0.05$. Decrease in SDH level was also observed in groups C_1 (1.81 ± 0.01), C_2 (1.50 ± 0.01) and F (1.99 ± 0.89) μmoles/min/mg respectively, as against the control group A and as compared with other groups at $P < 0.05$.

Table 1 shows an increase in the level of AChE activity in groups B_2 (4.63 ± 0.95), C_2 (8.12 ± 3.77), D (4.57 ± 0.26), E (11.46 ± 0.26) and F (13.50 ± 0.52) moles/min/gm respectively and this was significant at $P < 0.0001$*** when compared with each other in the experimental group at $P < 0.05$. A decrease in the level of AChE was observed in groups B_1 (0.56 ± 0.08) and C_1 (0.54 ± 0.02) moles/min/gm respectively at $P < 0.0001$*** when compared with group A and with other groups at $P < 0.05$.

The preservation of sufficient levels of acetylcholine (ACh) at neurotransmission sites is the basic plan of action involved in the medical treatment of neurodegenerative disorders such as Alzheimer's disease [23]. Consequently, the inhibition of acetyl cholinesterase (AChE) prevents the hydrolysis of ACh thereby maintaining normal memory function. Frequency of neurodegenerative disorders reduces in correlation to consumption of antioxidants [24]. Due to this, diets including natural compounds with high levels of antioxidants have been advocated as useful remedial approach for neurodegenerative disorders [24].

AChE ceases the action of acetylcholine post-synaptically [25]. The inhibition or introduction of AChE activity will result in alterations in the concentration of acetylcholine which may have a negative effect on the body. When AChE is inhibited in the body, high concentration of

acetylcholine will accumulate in the body while hydrolysis of the acetylcholine into acetate and choline occurs due to introduction of AChE in the body, this will reduce the concentration of the acetylcholine in the body [24]. The toxicity of acetylcholine is manifested by muscarinic and nicotinic signs and symptoms such as ocular pain, confusion, ataxia, slurred speech, loss of reflexes, generalized convulsions and coma [24].

There was a significant decrease in AChE activity in group B_1 and group C_1 animals' frontal cortex tissue. This finding supports previous research findings that the rate of AChE activity decreased significantly when compared with the normal control group A [25]. Another study reported that cadmium and alcoholic herbal beverages increases AChE activity in response to a dose per body weight of rat [26]. There was an increase in acetyl cholinesterase enzyme activity of group B_2, group C_2, group E and group F rats when compared to the cadmium induced group A rats, though it increased compared to the group B_1 and group C_1 rats. This shows the antioxidants properties of *Moringa oleifera* oil extract in restoring the action of AChE activity from very low levels to a significant high level. This is supported by previous researches that antioxidants are powerful therapeutic agents that can prevent neurodegenerative disorders, and a combination, rather single use of any of them is more effective [27]. This may also be the reason why there was a significant reduction in AChE activity in group D rats when compared to the normal group A animals. There was a significant increase in the level of activity of AchE in frontal cortex of group C_2 when it was compared to animals in the control group A, this observation was in line with report of [27]. In group F rats, that are the *Moringa oleifera* seed oil control group, there was a significant increase in Acetyl cholinesterase activity when compared with the control rats. This probably might be due to the antioxidant activity in the Moringa plant [28].

From the results of this study, there was an increase in the level of SDH activity in group B_1, group B_2 and group E rats which were significant at $P < 0.05$ when compared with control group A animals. This observation was in line with investigation carried out by [29]. There was a decrease in the level of SDH activity in the frontal cortex homogenates in group C_1, group C_2 and group F, this decrease in SDH activity is significant at $P < 0.05$ when compared with animal frontal cortex homogenate in group A which explained the importance of SDH at decreased level in Kreb's cycle to generate high- requiring ATP needed for brain cells.

Result of the activities of oxidative stress markers

Table 2 Effects of MO seed oil on oxidative stress markers in brain tissue homogenates of Rats

GROUPS	**A**	**B1**	**B2**	**C1**	**C2**	**D**	**E**	**F**
CAT (µmoles/mg)	1158±14.47	1331±23.12*	1456±49.45*	1355±60.68*	1397±0.74*	1209.±39.29	1797±41.17*	1807±67.34*
MDA (10-6 units/mg)	5.40±0.083	7.30±0.17*	42.36±1.49	20.79±0.89	15.37±0.11	22.94±0.82	55.40±1.19*	24.16±1.20*
GPx (µmoles/min/mg)	366.50±4.37	444.40±9.08*	466.80±16.1*	461.40±19.9	446.40±8.08	461.7±15.7	639.40±14.3	560.90±21.4
SOD (units/mg)	2796.00±6.11	83.33±0.00	666.70±0.00	116.70±0.00	785.70±30.73	78.57±3.07	83.33±0.00	3476.00±2.02

Data are expressed as means ± SEM * *It indicates significant level at* $P < 0.05$ *when compared with each other (multiple comparison test} and with the control group A.*

Legend: CAT: Catalase, MDA: Malondialdehyde, GPx: Glutathione Pereoxidase, SOD: Superoxide Dismutase, **A:** Control, $\mathbf{B_1}$: Cadmium only, $\mathbf{B_2}$: Cadmium and Moringa oil, $\mathbf{C_1}$: HAB only, $\mathbf{C_2}$: HAB and Moringa oil, **D**: Cd and HAB, **E**: Cd + HAB + MO, **F**: *Moringa oleifera* seed oil.

There was a progressive increase in the level of catalase activity in groups B_1, B_2, C_1, C_2, D, E and F as shown in Table 2, this progressive increase was significant at $P < 0.0001$*** when compared within the groups and with the control group A at $P < 0.05$.

A progressive increase in the level of malondialdehyde was observed in groups B_1, B_2, C_1, C_2, D, E and F as shown in table 2, the increase was significant at $P < 0.0047$** when compared with the control group A and with each other across the groups at $P < 0.05$.

There was an increase in the level of glutathione peroxidase in groups B_1, B_2, C_1, C_2, D, E and F as described in table 2, this increase was significant at $P < 0.0001$*** when compared with each other across the experimental groups and with the normal control group A at $P < 0.05$.

Result of the level of SOD in Brain Homogenate

There was a progressive decrease in the level of superoxide dismutase in groups B_1, B_2, C_1, C_2, D and E and an increase in the level of SOD in group F (3476.00 ± 2.02) units/min which was significant at $P < 0.0001$*** when compared with control group and with each other across the experimental groups at $P < 0.05$.

The activities of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase have been affected by cadmium. Studies have demonstrated these changes in enzyme activities [30, 31]. Under normal conditions, free radicals are formed in minute quantities and are rapidly scavenged by natural cellular defense mechanisms comprising of enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and catalase (CAT) and others. The activity of antioxidant enzymes such as CAT, SOD, and GST, can vary depending on the intensity and duration of the chemical stress applied to the organism in addition to the susceptibility of the exposed species [32].

Cadmium caused a significant increase in malondialdehyde (MDA) activity in Group B1 animals' brain tissue homogenates. It also produced a resultant increase in glutathione pereoxidate (GPx) and catalase (CAT) activities, while superoxide dismutase (SOD) activity decreased, though these increments were not significant and decrease in SOD activities was significant when compared with control group A animals [33] reported a significant increase in lipid peroxidation in animals exposed to cadmium. Superoxide dismutase (SOD) and catalase (CAT) activities were also reported to be decreased ($P < 0.05$ and $P < 0.005$) in the same experiment, whereas glutathione peroxidase (GPx) increased.

The enzyme activities of GPx, MDA and CAT increased in group B_2, compared to their activities in groups of rats induced with cadmium only. This suggests that the extracts have protective action against free radicals. Previous researches have shown that non enzymatic antioxidant components which consists of molecules such as alpha tocopherol (Vit. E), ascorbic acid (Vit. C), glutathione and beta-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions. Whereas, there was a significant decrease in SOD activity.

In group C_1, group C_2, group D and group E animals showed a significant increase in the activities of MDA, GPx and CAT, while there was a significant decrease in enzyme activity of SOD. This is in agreement with previous studies which reported that, *Moringa oleifera* contains antioxidant phytochemicals, such as vitamin C, betacarotene, lycopene and vitamin E all of which acts as antioxidant and subsequently decrease the consumption of these antioxidant enzymes to combat oxidative stress [34, 35, 36, 37]. In an earlier study, the presence of alkaloids, flavonoids, saponin, tannin, anthraquinones, and anthacyanosides in *Moringa oleifera seed oil* extract was reported [37]. Also, previous independent studies have reported that the protective actions of hepatoprotective medicinal plants are mediated by their flavonoids or alkaloids components or by their combination via antioxidant and free radicals scavenging activities [37]. The presence of these active biological principles may thus be accounting for the biological effect of *Moringa oleifera* extract and could be via antioxidant and/or free radicals scavenging activities. Group F rats treated with *Moringa oleifera* seed oil extract also showed significant increase in SOD, GPx and CAT activities and a significant decrease in MDA activity as compared with control group rats at $P < 0.05$.

Conclusion

This study has shown that *Moringa oleifera* oil extract has antioxidant properties that might have ameliorated morphological and biochemical damage caused by cadmium and herbal alcoholic beverages.

Ethical Approval

All experimental investigations were done in compliance with the guideline, as stated in the "Guide to the care and use of Laboratory Animals Resources" [42] and in accordance with guidelines stated in IACUC, OLAW, United Kingdom and in accordance with the approval of

College of Health Ethical and Research Committee, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

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