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Haematopathic Assessment of *Moringa oleifera* Seed Oil in Cadmium and Herbal Alcholic Beverage Induced Blood-Cells Damaged in Wistar Rats

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ABSTRACTS

This study was targeted at investing the effects of *Moringa oleifera* seed oil in cadmium and herbal alcoholic beverage induced haematological disorders in Wistar rats. The investigated haematological parameters included haematocrit (HCT), haemoglobin (HGB), lymphocytes (LYM), mean corpuscular haemoglobin concentration (MCHC), red and white blood cells counts (RBC & WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and pack cell volume (PCV). Exposure to both $CdSO_4$ and herbal alcoholic beverage were significantly ($P<0.05$) reduced the amounts of WBC and MCHC. MCV values increased at ($P<0.05$) after the *Moringa oleifera* seed oil treatment but MCV estimates with Cd exposure showed a decrease as compared with herbal alcoholic beverage with an elevation at ($P<0.05$) in (MCV). MCH levels increased in both herbal alcoholic beverage and *Moringa oleifera* seed oil ($P<0.05$) whereas PCV level increased in Cd and HAB at ($P<0.05$) as compared with control and treatment groups, while increased in (LYM) counts were observed at significant level ($P<0.05$) in Cd and HAB as compared with control and treatment groups. The exposed animals showed some haematological disorders in respects to the observed parameters in Cd and HAB, meanwhile, ameliorative properties were examined in line with the above results in the treatment groups with *Moringa oleifera*.

Key words: *Moringa oleifera*, Haematology, Herbal Alcoholic Beverage, Cadmium, Blood Cells

INTRODUCTION

Blood is the most important connective tissue, in which changes in metabolic processes are reflected, therefore, abnormal alteration in blood parameters are the reliable indicator of toxic effects of drugs, toxicant xenobiotics and diseases [(1)] The alterations in haematological changes serve as the earliest indicators of toxic effects on tissue[(2)] .Cadmium has been recognized as a biological toxicant. They are widely dispersed in the environment and are, with excessive levels, environmental toxic to humans [(3)]. Absorbed cadmium following oral ingestion is carried via blood to soft tissues. In this respect, the present study was designed to evaluate the toxicological effects of cadmium and their combination on the disruption of hematology in Wistar rats [(4)]. There are scanty and sporadic reports available regarding the effect of Cd simultaneously on the haematological as well as on the biochemical parameters in addition to the light and electron microscopy of the liver[(5,6)].

Cadmium (Cd) is a toxic metal in the environment, found in the soil, rock phosphate fertilizer and in tobacco plant. Cd is a highly accumulative toxicant with very long biological half-life [(6)] . Cadmium cannot penetrate the adult blood brain barrier (BBB), although it might diffuse across the BBB with the help of a vehicle such as ethanol [(7)]. Cadmium can effectively pass the BBB during the developmental stage in an organism and is more toxic in new-borns [(8)] . Cadmium LD_{50} of 5.7 mg/kg per body weight intraperitoneally on rats and once administered, it accumulates in different areas of the brain, induces lipid peroxidation and weakens the antioxidant defense[(8)] . In battery workers Cd-induced oxidative stress was demonstrated to cause amyotrophic lateral sclerosis due to reduced brain SOD activity [(7)].

Moringa oleifera L (Moringaceae) commonly known as Ben oil plant or drumstick plant in English language, *'Okwe oyibo'* in Igbo, *'Gawara' or 'Habiwal'* in Hausa, *'Adagba maloye'* or '*Ewe Igbale*' in Yoruba and 'Avi Anahu' in Ebira grows rapidly in most regions of Nigeria [(9)]. *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 [10] . 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 [11]. It is an exceptionally nutritious vegetable tree with a variety of potential uses [12]. 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 [9,11]. 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 [9]. In addition to its compelling water purifying powers and high nutritional value, *Moringa oleifera* is very important for its medicinal value [11].

The 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, hepatopiotective, antibacterial and are being employed for the treatment of different ailments in the indigenous system [13,14].

Herbal alcoholic beverages commonly called Ogogoro, alomo bitter, Opa eyin in Nigeria. It is locally manufacture and package, it is consumed locally by the general public for sexual enhancement and as stimulants [14]. Various investigation has revealed the deleterious effects of high percentage of alcohol (ranging from 17% to 70%) in most of the herbal alcoholic beverages [7]. Excess consumption of alcoholic beverages has been associated with high libido causing excess sexual enhancement and over stimulation has also been linked to excess herbal alcoholic consumption. Overall effects of herbal alcoholic consumption has been revealed to cause health hazard leading to soft tissues damage such as cardiovascular, lung, liver, kidney and brain [7].
The use of herbal therapies in the world is escalating, it is essential to be aware of clinical and adverse effects, doses and potential drug-herb interactions [4].An estimated 15 million adults are at risk for potential adverse interactions involving prescription of medications and herbs or vitamin supplements, yet most practicing physicians have little knowledge of herbal remedies or their effects [15].

Herbs and spices can presumably acquire metals during growth in contaminated soils- including contamination of plant material with soil [4]. High levels of toxic metals can occur in medicinal preparations when they are used as active ingredients as in the case of Pb and Hg in some Chinese, Mexican and Indian herbal medicine or when the plants are grown in polluted areas, such as near roadways or metal mining and smelting operations [16] .Use of fertilizers contaminated with Cd or Pd, pesticides contaminated with heavy metals (organic mercury or lead based pesticides) and contaminated irrigation water during the growing of herbs and spices may be a source of heavy metal contamination of the final products [17]. Literature abounds on the contamination of HAB with heavy metals such as lead, mercury, arsenic, cadmium and on cases of human poisoning resulting from such consumptions. However, some Indian herbal (Ayurveda) remedies, folk medicines, and homeopathic remedies are purposely adulterated with metals in the mistaken belief that they confer a health benefit to the users [4]. Heavy metals are often found in herbal as they are thought to help cure without being absorbable. Heavy metal contamination is not uncommon in Asian herbal products. 24 of 251 Asian patent medicines collected from herbalstores in California, USA contained lead (at least 1ppm); 36 products contained arsenic, and 35 contained mercury [18].

Herbs can also be contaminated in the course of the milling or other processing procedures or in the extraction processes [15]. In the indigenous/traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extracts. Fresh plant parts, juice or crude powder are a rarity rather than the rule. The use of contaminated water or ethanol in such extraction processes can result in the contamination of the finished products [14]. In Nigeria, most locally produced, cheap and readily available alcoholic products are prepared either from the dilution of industrial grade alcohol or from the crude distillation of locally fermented palm wine, commonly referred to as *ogogoro* or *illicit gin* [14]. There may be serious contamination in these processes as they are rarely carried out under hygienic conditions [15]. The water used in these processes is readily obtained untreated either from boreholes or from natural surface water bodies which may have become contaminated from the metal contents of such geological strata, industrial effluents or solid wastes disposed into surface water bodies [19]. Sometimes the active ingredients of herbal therapies are substituted in part or completely with other compounds that may or may not contain the same active ingredient or the same curative properties. Additives may also be used but not listed on the label. Adulterants can also be added by unethical herbalists compounding preparations for individual patient [20].

MATERIALS AND METHODS

Moringa oleifera: *Moringa oleifera seeds were harvested from Baba Wali Street NTA Community, Behind Kogi State University Anyigba, Nigeria,* in June, 2019 *and the plant specimen was identified with voucher number (No.* KSU/BS/462*) assigned at* Department of Biological Sciences, Kogi State University, Anyigba*, by* Mr. Ayegba Ojochele Sule*, where it was deposited.*

Oil Extraction and Preparation: The seeds of fresh *Moringa oleifera* plant were plucked and air dried under room temperature at (29°C-35°C) for four (4) weeks, after which the seeds were pulverized into coarse form with Acrestor high speed milling machine. The coarse form (200g) was macerated in absolute ethanol. This was left to stand for 24hrs [21]. After that the extract was filter through muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract were concentrated and evaporated to dryness using rotary evaporator at an optimum temperature which was between 40^0C and 45^0C to avoid denaturation of the active ingredients. The concentrated extract was store in the refrigerator until use [21].

Determination of Yield of Extract: The percentage yield of the extract was determined by weighing the coarse *Moringa oleifera* seed before extraction and the *Moringa oleifera* ethanol seed extract after concentration and calculated using the formula [22].

$$\text{Percentage (\%) yield} = \frac{\text{Weight (g) of concentrated extract}}{\text{Weight of grounded Moringa Seeds}} \times 100$$

Determination of Phytochemicals: The quatitative phytochemical analyses was carried out according to the methods of [22,23].

Source of Cadmium and Herbal Alcoholic Beverage: A white powdery Cadmium sulfate octahydrate ($3CdSO_4 .8H_2O$) with molar mass M=769.52 and net weight W=100g was purchased in June, 2019 from Guangzhou linhuada Chemical Reagent Co. Ltd., Guangdog, China (Tel: 020-84382388, E-mail Adress: sches@-h-d.com). Reagent Lot No: 20120524 and Herbal alcoholic beverage was procured locally in Nigeria.

Administration of Chemical and Animal Treatment: The Cadmium stock solution was made by dissolving 11.27mg of Cadmium sulphate salt in 5.64ml of 0.9% w/v phosphate buffer at PH 7.4. The cadmium stock solution was administered intraperitoneally in doses corresponding to the weight of the rats using 1ml insulin syringes.

Animal Care: All experimental investigations were done in compliance with the guideline, as stated in the "Guide to the care and use of Laboratory Animals Resources" (*NRC, 2018*) and in accordance with guidelines stated in IACUC and OLAW, United Kingdom.

Conditioning of Animals: Animals were bred in the animal house of the College of Health Sciences, Kogi State University, Anyigba, Nigeria, to rule out the genetic effects on the investigation and second filial generation were used for the study. The study was carried out using healthy adult Wistar rats of both sexes weighing 75g – 145g. The animals were maintained under standard laboratory conditions of light, temperature, humidity and ventilation. They were given rat chow and water *ad libitum* and the experimental animals were acclimatized for two (2) weeks before the commencement of the research work.

Animal Grouping: A total of 56 adult Wistar rats aged eight (8) weeks of both sexes were used for this study. The animals were randomly divided into eight (8) groups A, B1, B2, C1, C2, D, E and F of Seven (7) animals each (Table 1).

Animal Treatment: The control group (A) received 2.5 mg/kgbw of phosphate buffer intraperitoneally single dose and the induced control group (B1 and B2) received 3.5 mg/kgbw of $3CdSO_4.8H_2O$ (Ige *et al.,* 2010) intraperitoneally and left for 72 hours. B_1 rats were maintained under normal laboratory condition for 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 [24] following oral administration of 0.5 ml, 40% Herbal-gin single dose daily for the period of four weeks. 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[24] 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.

Table 1: Experimental Design for Animal Treatment

Animal Grouping	NO of Animals	Animal Induction/Treatment
Group A	7	2.5 mg/kgbw Buffered Phosphate solution
Group B1	7	3.5 mg/kgbw $CdSO_4$ only
Group B2	7	3.5 mg/kgbw $CdSO_4$ + 2.0mg/kgbw *Moringa oleifera* oil
Group C1	7	0.5 ml, 40% Herbal alcoholic beverage only
Group C2	7	0.5 ml, 40% HAB + 2.0mg/kgbw *Moringa* oil
Group D	7	3.5 mg/kgbw $CdSO_4$ + 0.5 ml, 40% HAB
Group E	7	3.5 mg/kgbw $CdSO_4$ + 0.5 ml, 40% HAB + 2.0 mg/kgbw *Moringa-oleifera* oil
Group F	7	2.0 mg/kgbw *Moringa oleifera* oil only

Animal Sacrifice: 24 hours after the last administration, all animals were sacrificed via cervical dislocation.

Blood Collection and Haematological Analysis: After 28 days of exposure, rats were fasted overnight. They were weighed before the collection of blood and sacrifice. All samples were taken between 7 and 9 am to avoid variations due to circadian rhythm. Whole blood was obtained from a puncture of the retro-orbital sinus by the conventional method [(7)].

Blood samples collected in ethylene diamine tetra-acetic acid (EDTA) anticoagulant tubes (8.5%) was quickly returned by mixing with anticoagulant in the tube. All blood samples were labeled and immediately conveyed to the laboratory for analysis. Hematological parameters were analyzed: white blood cell count (WBC), red blood cells (RBC), hemoglobin concentration (HGB), haematocrit (HCT), the mean corpuscular hemoglobin (MCH), volume a mean corpuscular erythrocyte (MCV), the mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) and the number of lymphocytes (LYM) [(25)]. All hematological parameters were analyzed in the "Medical Biochemistry Laboratory, Faculty of Basic Medical Sciences, College of Health Sciences, Kogi State University Anyigba" using the automated method with the automatic analyser "Haematology auto analyzer Sysmex KX-21N".

Statistics Analysis: Data were subjected to analyses using Microsoft excel, SAS 9.12 and GraphPad Instat software. One -way analysis of variance (ANOVA) was used to detect the treatment effects. Pearson correlation was used to evaluate relationship between measured parameters. The means were separated by Duncan multiple range test (DMRT), mean with same superscript in the same row are not significantly different and a probability value < 0.05 was considered statistically significant.

RESULTS

Result of the Percentage Yield of *Moringa oleifera* Seed Oil Extract: A total percentage of 33.1 % oil yield was obtained from 200 g of dried coarse form of the *Moringa oleifera* seed used in this study. The 33.1 % oil yield was displayed in three layers comprising of (a) the top layer presentation of light yellow colour spectrum (24.1 %), (b) middle layer of thick yellow – milky spectrum (2.2 %) and (c) the lower layer of reddish brown colour spectrum (6.8%) as shown in Table 2.

Table 2: Result of the percentage Yield of *Moringa oleifera* Seed

Moringa oleifera seed oil	Colour Spectrum Layers	Percentage yield of *Moringa* oil ((%)
Top layer	Light Yellow	24.1
Middle layer	Thick Yellow -Milky (as fats)	2.2
Lower layer	Reddish Brown	6.8
Total (%)		33.1

Phytochemical Analysis of *Moringa Oleifera* Seed Powder and Seed Extract: The *Moringa oleifera* seed powder and the Moringa extract showed high quantity of alkaloids, carbohydrate quantity was high in *Moringa* seed extract. Flavonoids, Steroids and Cardiac glycosides were in abundance in both the *Moringa* seed powder and seed extract. Saponins were present in the *Moringa* seed power but absent and tested negative in the Moringa seed extract while tannins were confirmed absent in both the seed powder and seed extract as shown in table 3.

Table 3: Phytochemical Analysis of *Moringa oleifera* Seed Powder and Oil Extract

Bioactive Constituents	Moringa Seeds Powder	Moringa Seed Extract
Alkaloids	+ + +	+ + +
Saponins	+	-
Tannins	-	-
Flavonoids	+	+
Carbohydrates	+ +	+ + +
Steroids	+ +	+ +
Anthraquinins	-	-
Cardiac glycosides	+	+ +

Key: - Negative, + Present, + + More Present, + + + Highly Present

Table 4: Shown Assessment of Haematological Parameters in Cd and HAB Treated with *Moringa oleifera* Seed Oil in Wistar Rats

PARAMETERS	GRP A	GRP B_1	GRP B_2	GRP C_1	GRP C_2	GRP D	GRP E	GRP F
WBC × 10^3	7.74 ± 0.544^{d}	5.500 ± 0.100^{h}	10.26 ± 0.207^{b}	5.80 ±0.148^{g}	10.80 ± 0.236^{a}	7.80 ± 0.188^{c}	7.29 ± 0.125^{f}	7.60 ± 0.476^{e}
RBC × 10^6	6.65 ± 0.333^{e}	6.741 ± 0.384^{d}	7.33 ± 0.143^{b}	3.69 ± 0.290^{h}	8.35 ± 0.087^{a}	5.61 ± 0.496^{g}	6.98 ± 0.263^{c}	6.20 ± 0.337^{f}
HGB	12.84 ± 0.401^{c}	11.21 ± 0.432^{h}	12.67 ± 0.253^{d}	11.36 ± 0.518^{g}	15.47 ±0.109^{a}	11.84 ± 0.321^{f}	13.36 ± 0.309^{b}	12.33 ± 0.231^{e}
HCT/PCV	40.03 ± 1.329^{e}	41.91 ± 2.304^{d}	43.97 ± 0.681^{c}	25.31 ± 1.660^{h}	51.23 ± 0.337^{a}	37.53 ± 0.604^{f}	46.30 ± 0.432^{b}	37.21 ± 1.802^{g}
MCV	64.27± 1.329^{d}	62.03 ± 0.324^{f}	62. 47 ± 0.639^{e}	69.83 ± 1.878^{a}	62.69 ± 0.587^{e}	65.44 ± 0.536^{c}	60.97 ± 0.261^{g}	66.16 ± 1.581^{b}
MCH	21.44± 0.803^{b}	18.73 ± 0.499^{f}	18.73 ± 0.201^{f}	35.06 ± 2.331^{a}	18.86 ± 0.115^{e}	20.34 ± 0.463^{d}	17.71 ± 0.191^{g}	20.66 ± 1.275^{c}
MCHC	32.16± 0.796^{b}	31.17 ± 0.711^{c}	29.77 ± 0.245^{e}	45.47 ± 3.065^{a}	30.49 ± 0.472^{d}	29.57 ± 0.658^{e}	30.56 ± 0.359^{d}	32.03 ± 1.455^{b}
LYM %	85.30 ±2.821^{b}	83.69 ± 1.381^{c}	64.69 ± 0.320^{d}	77.39 ± 1.751^{d}	64.27 ± 0.668^{d}	86.21 ± 1.385^{a}	83.70 ± 0.775^{c}	76.17 ± 2.952^{e}
RDW_SD	48.64± 1.834^{d}	42.69 ± 0.505^{f}	44.10 ± 0.703^{e}	52.27 ± 1.788^{b}	42.96 ± 0.456^{f}	50.43 ± 0.644^{c}	41.97 ± 0.346^{g}	58.63 ± 2.898^{a}
RDW_CV	20.27± 0.660^{d}	18.44 ± 0.289^{f}	17.86 ± 0.248^{g}	29.07 ± 1.372^{a}	16.97 ± 0.164^{h}	20.96 ± 0.560^{c}	18.89 ± 0.187^{e}	22.16 ± 1.295^{b}
PDW	8.20± 0.293^{e}	9.27 ± 0.094^{c}	8.97 ± 0.201^{d}	9.11 ± 0.124^{cd}	7.94 ± 0.209^{f}	10.39 ± 0.116^{a}	9.27 ± 0.089^{c}	9.56 ± 0.450^{b}
MPV	6.66± 0.172^{fg}	7.26 ± 0.129^{d}	7.43 ± 0.121^{b}	6.99 ± 0.174^{e}	6.77 ± 0.225^{f}	7.64 ± 0.065^{a}	7.37 ± 0.087^{c}	7.34 ± 0.125^{cd}
P_LCR	6.86 ± 0.629^{h}	9.54 ± 0.432^{d}	9.47 ± 0.337^{e}	13.06 ± 0.298^{a}	7.53 ± 0.719^{g}	12.60 ± 0.136^{b}	8.56 ± 0.111^{f}	10.76 ± 0.845^{c}
PLT/µL × 10^5	414.14 ± 62.960^{h}	573.00 ± 131.930^{f}	698.00 ± 28.577^{c}	576.86 ± 95.187^{e}	713.14 ± 111.570^{b}	598.47 ± 136.890^{d}	883.29 ± 30.574^{a}	532.43 ± 54.717^{g}
LYM/µL × 10^3	7.03± 0.382^{e}	11.50 ± 6.602^{a}	7.11 ± 0.325^{d}	6.50 ± 0.242^{f}	7.80 ± 0.216^{b}	6.04 ± 0.281^{g}	6.07 ± 0.277^{g}	7.24 ± 0.489^{c}

Key: n = 7, mean ± SEM

Morphometric Analysis- Average Body Weight: The animals were observed and the weighed of the Wistar rats were recorded on alternate days for four weeks. The result showed that there was a significant increase in the average body weight of the Wistar rats upon treatment with *Moringa oleifera* seed oil in groups B_2, C_2 and E (149.60 ± 13.30 g, 124.50 ± 5.13 g and 136.50 ± 3.16 g) respectively. This increase in the average body weight was at p< 0.0048 which was statistically significant when compared and tested with each other at p< 0.05. a decrease in average body weight was observed in groups B1, C1, and D rats (124.30 ± 7.77 g 105.40 ± 2.84 g, 127.70 ± 8.27 g) respectively and this decrease in the average body weight was at p< 0.0048 which was significant at p< 0.05 at both multiple comparism test and as compared with the normal control group A rats as shown in table 5.

Relative Brain Weight (RBW): A significant increase in the relative brain weight was observed in groups B_1, C_2, E and F (0.86 ± 0.03 g, 0.86 ± 0.08 g, 0.95 ± 0.02 g and 0.81 ± 0.02 g) respectively, these increase was at p<0.0012 which was significant at p< 0.05 when compared with each other and with the normal control group A, significant difference was not observed in relative brain weight in groups B1 and C1 (0.80 ± 0.02 g, 0.79 ± 0.03 g) respectively when compared with the normal control group A, but were significant at p< 0.0012 when multiple comparism test was carried out at p< 0.05. Group D showed a decrease in relative brain weight (0.54 ± 0.04 g) when compared with the normal control group A and with other groups at p< 0.0012 and tested statistically significant at p< 0.05 as observation in table 6.

Table 5: The Average Animal Body Weight of Rats Induced with Cd and HAB Treated with *Moringa oleifera* Seed Oil

DURATION	GROUP (n = 10) ABW (g)								
	A	B1	B2	C1	C2	D	E	F	P-Value
WK1	79.80±3..74	82.80 ±0.72	88.30±1.81	73.90±2.98	76.50±3.46	75.40±1.95	81.40±4.27	81.80±4.02	0.0378
WK2	84.90±5.40	99.10±3.68	106.10±3.51	82.20±2.72	92.70±5.07	90.40±4.02	110.70±7.81	103.50±7.03	0.0125
WK3	134.30±7.34	`127.00±8.36	138.10±12.48	115.60±2.88	107.80±3.94	136.90±8.34	135.00±4.29	131.80±6.05	0.0340
WK4	151.00±2.94	124.30±7.77*	149.60±13.30*	105.40±2.84*	124.50±5.13	127.70±8.26*	136.50±3.16	149.40±7.17*	0.0048

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: WK: Week, A: Control, B1: Cadmium only, B2: Cadmium and *Moringa* oil, C1: HAB only, C2: HAB and *Moringa* oil, D: Cd and HAB, E: Cd + HAB + MO, F: *Moringa oleifera* seed oil

Table 6: The Relative Brain Weight of Rats Induced with Cd and HAB Treated with *Moringa oleifera* Seed Oil

DURATION	GROUP (n = 10) RBW(g)								
	A	B1	B2	C1	C2	D	E	F	P-Value
WK4	0.79±0.03	0.80±0.02	0.86±0.04*	0.79±0.03	0.86±0.01*	0.54±0.04*	0.95±0.02*	0.81±0.02	0.0012

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: WK: Week, A: Control, B1: Cadmium only, B2: Cadmium and *Moringa* oil, C1: HAB only, C2: HAB and *Moringa* oil, D: Cd and HAB, E: Cd + HAB + MO, F: *Moringa oleifera* seed oil

DISCUSSION

The use of plants with medicinal properties for the treatment, cure and prevention of diseases is one of the oldest medicinal methods known in history. At the beginning of the 1990s, the World Health Organization stated that 65-80% of the population of developing countries depend on medicinal plants as their only form of basic health care [26].

Cadmium (Cd) is known to produce a variety of health hazards in humans and experimental animals due to its ability to induce severe alterations in various organs and tissues including the nervous system, following either acute or chronic exposure [8] promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions [8] .This investigation examined the intervention of *Moringa oleifera* seed oil extract in cadmium and herbal alcoholic beverage induced damage to the Wistar rats, which involved haematological studies.

The table 4 above shows haematological parameters included haematocrit (HCT), haemoglobin (HGB), lymphocytes (LYM), mean corpuscular haemoglobin concentration (MCHC), red and white blood cells counts (RBC & WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and pack cell volume (PCV). Exposure to both $CdSO_4$ and herbal alcoholic beverage were significantly ($P<0.05$) reduced the amounts of WBC and MCHC. MCV values increased at ($P<0.05$) after the *Moringa oleifera* seed oil treatment but MCV estimates with Cd exposure showed a decrease as compared with herbal alcoholic beverage with an elevation at ($P<0.05$) in (MCV). MCH levels increased in both herbal alcoholic beverage and *Moringa oleifera* seed oil ($P<0.05$) whereas PCV level increased in Cd and HAB at ($P<0.05$) as compared with control and treatment groups, while increased in (LYM) counts were observed at significant level ($P<0.05$) in Cd and HAB as compared with control and treatment groups, is in line with [27].

The gross morphology of the rats across the experimental groups revealed a variation which encompassed a decrease and increase in the average animal body weight as well as relative brain weight. A significant decrease in both animal average body weight and relative brain weight were observed in cadmium and herbal alcoholic beverage treated groups, this observation is synanymous with the report of [28] that cadmium and herbal gin are associated with organs weight lost. An increase in the average weight and relative brain weight were also observed upon administration of *Moringa oleifera* seed oil, this observation also corroborate the reported study by [29] that antioxidants in *Moringa oleifera* plant are capable of ameliorating the effects of free radicals in cell and tissue.

CONCLUSION

This study has revealed the following:

Moringa oleifera seed oil extract has high percentage (33.1%) yield of oil as well as natural antioxidants useful to the body system in maintenance and building of immune defense mechanism against stress and factors causing diseases.

It has been found that *M. oleifera* seed oil has ameliorative effects on morphological damage caused by cadmium and herbal alcoholic beverage.

RECOMMENDATION

1. *Moringa oleifera* seed oil should be subjected into more studies involving clinical trials.
2. *Moringa oleifera* seed oil efficacy and medicinal importance should be advocated and more public awareness should be encouraged on the use of the plant extract.
3. Separate dwelling spaces from areas where Cadmium are used as main source of raw materials. In particular, isolate children from indoor exposure to Cadmium emission.
4. Avoid domestic use of Cadmium containing products such as tobacco leaves and prohibit smoking of cigarette in a closed environment.
5. Production and sales of Herbal Alcoholic Beverages should be regulated, proper and regular monitoring system should be encouraged.
6. Raise public awareness regarding sources of exposure to Cd and consumption of herbal alcoholic beverages stating the risk mitigation measure.
7. Conduct educational activities to discourage the use of Cd-containing products and consumption of Herbal Alcoholic Beverages.
8. Adequate and good monitoring and workable health system should be made available and accessible to workers or people living in areas where exposure to Cd is very high.
9. Research in phytochemical therapy for neurological diseases should be encouraged and government should made more financial aids available for researchers in this areas of study.

COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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