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Effect of African Walnut (*Tetracarpidium Conophorum* ((Müll. Arg.)) Hutch & Dalziel Syn. *Plukenetia Conophora*) Oil On Cadmium -Induced Oxidative Stress in Male Albino Rats

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ABSTRACT

This study was designed to investigate the protective effect of walnut oil against cadmium-induced oxidative stress in albino rats. 35 male rats were divided into 5 groups of 7 animals each. All groups were fed normal rat chow and distilled water or cadmium-poisoned water (200 ppm of cadmium as cadmium chloride) for 4 weeks *ad libitum*. Groups 1 (control) and 2 received distilled water and cadmium-poisoned water respectively. Groups 3 and 4 received cadmium-poisoned water and 2.0g/kg and 4.0g/kg body weight of walnut oil respectively by oral intubation. Group 5 was given distilled water and 2.0g/kg body weight of walnut oil for 4 weeks. The cadmium exposure resulted in oxidative stress in the rats expressed by the significantly increased organ weights of the test animals compared to the control animals, increased catalase activities in the liver and kidney of cadmium-exposed animals and increase in lipid peroxidation in liver, kidney and brain tissues. Administration of walnut oil at 2.0g/Kg body weight reversed these conditions towards control values and also led to a decrease in blood cadmium levels. Walnut oil at 4.0g/Kg body weight was not as effective as the lower dose.

Keywords: *Tetracarpidium conophorum*, cadmium, oxidative stress, antioxidants

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INTRODUCTION

A lot of research interest has been directed in recent years to heavy metal toxicity both in plants and animals [1-4]. Research into the use of plant based extracts in the management of heavy metal toxicity is also gaining global interest because of the numerous side effects associated with the use of traditional metal chelators [5-8].

Plants rich in antioxidants, a group of compounds with quite different chemical structures, are able to neutralize free radicals generated in heavy metal toxicity and during the aging process and have a potential role in preventing the onset of some chronic diseases such as cardiovascular disease, some neurological disorders or certain inflammatory processes. [9]. African walnuts, *Tetracarpidium conophorum* (Müll. Arg.), found to be rich in antioxidants and essential nutrients [10], is an example of such plants. *T. conophorum* is a climber found in the wet part of Southern Nigeria and West Africa. The fruits are greenish with four round seeds in each fruit. The seed testa is hard and the cotyledons are white in colour [11]. Several works have found different parts of this plant to have antioxidant properties [12]. *T. conophorum* belongs to the class of plant foods, particularly nuts, which have a high fat content and at the same time exhibit considerable antioxidant capacity. Some recent research has determined, through various different methodologies, the antioxidant capacity of different nuts, such as almonds, Brazil nuts, hazelnuts, macadamias, peanuts, pecans, pine nuts, pistachios, and walnuts [13-14. These studies concluded that walnuts possessed the greatest antioxidant capacity than the other nuts and that this antioxidant property is presumably a product of phenolic compounds, especially hydrolysable tannins, tocopherols and, according to some recent works also melatonin, an indoleamin that exhibits high antioxidant capacity.

Heavy metals such as cadmium, lead, mercury and arsenic threaten human health both through environmental and occupational exposure. Many factors such as smoking, air pollution and consumption of fruits and vegetables grown with uncontrolled use of fertilizers lead to chronic cadmium toxicity [15]. Like other heavy metals, cadmium generates reactive oxygen species (ROS), followed by development of oxidative stress in the target organs. This is one of several mechanisms through which cadmium exerts its toxicity [16].

The present study was conducted to investigate the effect of cadmium on some markers of oxidative stress in the tissues of male albino rats, with special reference to the possible beneficial role of walnut oil in ameliorating the cadmium-induced toxicity in the rats.

MATERIALS AND METHODS

Experimental Animals

Thirty-five male albino rats (Wistar strain) weighing between 120-150g were used for the study. The animals were obtained from the animal house of University of Ibadan, Oyo State, Nigeria. The rats were divided into 5 groups of 7 animals each and kept in separate cages in the animal house of Bells University of Technology, Ota and acclimatized for 2 weeks under normal

environmental conditions with 12 hour light/dark cycle. They were fed normal rat chow and water *ad libitum.* Rat weight was measured with a laboratory electronic scale, which is accurate to within 0.01grams.

Extraction of walnut oil

The walnuts were purchased from Oja Titun market in Ife, Osun State in Nigeria and were authenticated by Dr. P. I. Oni of the Biological Sciences Department of Bells University of Technology, Ota, Ogun State. Walnuts were separated from their shells, air-dried and milled. Portions of the pulp (about 40g) were extracted at a time with n-hexane using a Soxhlet apparatus. After extraction the solvent was removed yielding the oil. Any remaining solvent in the oil was removed by gentle evaporation over a water bath at 60°C. The oil was stored in the fridge at about 4°C until used. When needed the oil was brought to room temperature before administration.

Experimental Protocol

The five groups of rats were treated as follows:

Group 1: served as control and received distilled water (Cadmium-free) and normal rat chow.
Group 2: was given 200ppm of cadmium as cadmium chloride in their drinking water and normal rat chow.
Group 3: same as group 2, plus 2.0g/kg body weight of walnut oil daily by oral intubation.
Group 4: same as group 2 plus 4.0g/kg body weight of walnut oil daily by oral intubation.
Group 5: same as group 1 plus 2.0g/kg body weight of walnut oil daily.
Treatment was administered for a period of 4 weeks.

Experimental Animals

Daily weights of the rats were taken during the experiment. At the end of four weeks, the rats were fasted overnight and sacrificed by cardiac puncture under light ether anaesthesia. The brain, kidney and liver tissues were quickly removed, washed in ice cold 1.15% KCl solution, blotted dry and weighed. They were then homogenized in 4 volumes of the homogenizing buffer (phosphate buffer, pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 7000 g for 20 mins, the supernatant decanted and stored in a freezer at -4^{o}C. The above steps were carried out at temperatures between 0-4^{o}C. Aliquots of blood samples were preserved for cadmium analysis.

EXPERIMENTAL

Determination of Catalase Activity in the Liver and Kidney

Microsomal catalase activity was determined according to the method of Sinha [17]. This method is based on the reduction of dichromate in acetic acid to chromic acetate when

heated in the presence of hydrogen peroxide, with the formation of perchromic acid as unstable intermediate. The chromic acetate so produced is measured colorimetrically at 570nm. Catalase preparation (in samples) is allowed to split hydrogen peroxide for about one minute. The reaction is stopped afterwards by the addition of dichromate-acetic acid mixture and any hydrogen peroxide which hasn't been split by the catalase will react with the dichromate to give a blue precipitate of perchromic acid. This unstable precipitate is then decomposed by heating to give the green solution which is read spectrophotometrically.

Assessment of Lipid Peroxidation in the Liver, Kidney and Brain.

Lipid peroxidation in post mitochondrial fraction was estimated spectrophotometrically by thiobarbituric acid reactive substance (TBARS) in the brain, liver and kidney by the method of Tanero [18]. The principle is based on the reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA), an end product of lipid peroxidation. On heating in acidic pH, a pink coloured complex is formed as a product which absorbs maximally at 532nm. The absorbance is read spectrophotometrically and the result expressed as the amount of free MDA produced per gram wet weight of tissue.

Deteremination of Cadmium in the Blood

Blood cadmium was determined by atomic absorption spectrometry (Thermo Scientific Equipment S-series (model S4 AA system)). Concentrated nitric acid was used for the digestion of the blood samples which was then read with the AAS.

STATISTICAL PROTOCOL

Results are expressed as mean ± S.D. One way analysis (ANOVA) followed by Duncan's test was used to analyse the results with $p<0.05$ considered significant.

RESULTS AND DISCUSSION

Table 1 shows the result of the mineral analysis carried out on the walnut oil. The oil is rich in vital minerals such as sodium, potassium, calcium and iron. These are ions that have been shown to compete with cadmium for absorption across the intestinal epithelial cells. This might account for the reduction in cadmium absorbed in the animals administered walnut oil in this study. Cadmium was below detection limit in the oil but there was a trace amount of lead present. Manganese and copper, two minerals that are essential cofactors in the antioxidant enzyme superoxide dismutase (SOD), are also present in the oil. SOD disarms free radicals produced within cell cytoplasm and the mitochondria (the energy production factories within our cells).

Table 1: Result of Mineral Analysis on Walnut Oil Using Atomic Absorption Spectroscopy

Minerals	Amount present in oil (mg/l)
Na	98
K	148
Cu	0.1
Co	0.8
Fe	5.3
Ca	111.8
Mg	7.4
Al	11.05
Ni	0.15
Pb	2.15
Cd	ND

The percentage weights of the liver, kidney and brain of the animals are shown in Table 2. There is a significant increase ($p<0.05$) in the percentage weights of the three organs in the cadmium-exposed animals compared with control animals. These findings are in agreement with previous reports demonstrating that heavy metal toxicity leads to abnormal body and organ weights [3, 19]. Walnut oil at 2.0g/Kg body weight reduced this observed increase towards control values more efficiently than the higher dose. The higher dose of walnut oil administered appeared to stress the animals further as evidenced in the statistically significant ($p<0.05$) increase in percentage organ weights of the animals above both control and cadmium-exposed group. Group 5 (walnut only group) showed a significant increase in percentage weight of the brain above control values. This could be attributed to the presence of trace amounts of lead found in the oil. Lead is another well-known heavy metal and neurotoxin [20]. We postulate that in the absence of cadmium in this group, the trace lead was more absorbed.

Table 2: The Percentage Weight of the Organs

Groups	Liver wt.(%)	Kidney wt. (%)	Brain wt. (%)
Control	3.19 ± 0.16a	0.70 ± 0.04a	0.78 ± 0.14a
Group 2	3.65 ± 0.07b	0.86 ± 0.04c	1.11 ± 0.08c
Group 3	3.45 ± 0.27b	0.76 ± 0.04b	1.04 ± 0.03bc
Group 4	4.00 ± 0.13c	0.96 ± 0.02d	1.46 ± 0.05d
Group 5	3.22 ± 0.19a	0.71 ± 0.04ab	0.96 ± 0.09b

Values are Mean ± standard deviation (SD). Values in a column having no letter in common (a-c) in common are significantly different from each other ($p<0.05$)

Table 3 shows catalase activities in the liver kidney and brain of the animals. Catalase activities in groups 2, 4 and 5 of the liver was significantly increased ($p<0.05$) compared to control values. Walnut oil administered at 2.0g/Kg body wt seemed to be more effective in

lowering this cadmium-induced increase in catalase activity compared to the 4.g/Kg body wt. Administration of walnut oil alone also resulted in a significant increase in catalase activity as seen in group 5 result showing that the walnut oil alone led to a measure of oxidative stress in the animals. In the kidney, cadmium-treated rats showed a significant increase ($p<0.05$) in activity of catalase compared with control. Walnut oil treated rats also showed a significant increase ($p<0.05$) in catalase activity. Administration of walnut oil at the two different concentrations led to a reduction of catalase activity towards control values.

Table 3: Catalase Activities in the Liver and Kidney.

Group	Liver(µmol/ sec / g wet tissue)	Kidney(µmol/ sec / g wet tissue)
Group 1 (Control)	7.11 ± 1.20ab	4.77 ± 1.05a
Group 2	11.19 ± 2.04c	6.26 ± 0.34b
Group 3	6.05 ± 0.86a	5.82 ± 0.77ab
Group 4	8.63 ± 1.51b	5.43 ± 0.82ab
Group 5	10.58 ± 0.89c	6.34 ± 1.44b

Values are Mean ± SD. Values in a column having no letter in common (a-c) in common are significantly different from each other ($p<0.05$)

Table 4 shows the level of lipid peroxidation in the liver, kidney and brain estimated as the amount of malondialdehyde present in the different tissues. In the liver, there is a significant increase in lipid peroxidation ($p<0.05$) in groups 2, 3 and 4 compared to the control group, there was no difference in the walnut administered group. There is no significant difference ($p<0.05$) in lipid peroxidation levels between groups 5 and control. In the kidney, there is a significant increase ($p<0.05$) in lipid peroxidation levels in the cadmium exposed group compared to the control group. Administration of walnut oil reversed the observed increase in lipid peroxidation of group 3 and 4 towards the control group. There is no significant difference in lipid peroxidation levels of group 5 as compared to the control values. In the brain, there is significant increase ($p<0.05$) in lipid peroxidation in group 2 as compared to the control group. However, administration of the walnut oil restored this observed increase in lipid peroxidation levels in group 3 and 4 animals towards control group. There is no significant difference ($p<0.05$) in group 5 compared to the control group.

Table 4: Lipid Peroxidation as levels of malondialdehyde (MDA) in the tissues

Groups	Liver(nmol MDA/gwet tissue)	Kidney(nmolMDA/g wet tissue)	Brain(nmolMDA/gwet tissue)
Group 1 (Control)	84.1216.76a	87.84 ± 15.37a	51.89 ± 5.61a
Group 2	110.11 ± 13.66b	105.30 ± 9.22b	69.14 ± 6.39b
Group 3	109.23 ± 5.99b	94.41 ± 8.20ab	51.50 ± 16.63a
Group 4	120.00 ± 13.59c	99.62 ± 7.77ab	43.49 ± 7.47a
Group 5	95.63 ± 5.48ab	99.11 ± 8.83ab	50.26 ± 4.04a

Values are Mean ± SD. Values in a column having no letter in common (a-c) in common are significantly different from each other ($p<0.05$)

Table 5 shows the blood levels of cadmium in the animals. There was a non-statistically significant ($p<0.05$) accumulation of cadmium in the cadmium - exposed animals as compared with the control group. Walnut oil administered at both concentrations led to a reduction in the observed accumulation of cadmium towards control values. There was no significant difference ($p<0.05$) between cadmium levels in control group and walnut oil group. Some other studies have observed chelating properties in the aqueous extract of the seed [21]. In this study the oil could have served as a chelator of cadmium or the ions abundant in the oil could have served as selective competitive inhibitors of cadmium absorption across the intestinal epithelial cells.

Table 5: Blood cadmium levels in the animals.

GROUPS	BLOOD Cd (µg/ml)
Group 1	13.57±0.43a
Group 2	15.26±0.43a
Group 3	12.36±2.15a
Group 4	12.80±0.66a
Group 5	13.64±1.85a

Values are Mean ± SD. Values in a column having no letter in common (a-c) in common are significantly different from each other ($p<0.05$)

These results suggest that cadmium-induced oxidative stress in different organs could be managed variably by the nutritional benefits of walnut oil. However, it is observed that the dosage of the oil is vital to harnessing its antioxidant property. This is in consonance with other works that have found tree nuts to be effective antioxidants; lowering cholesterol levels, improving endothelial function, lowering oxidation in blood and reducing lipoprotein levels [13, 22]

Zinc, selenium, carotenoids and vitamins are among the important non-enzymatic antioxidant species [23]. Walnut oil has been shown to be rich in vitamin E, and vitamin E aids free radical destruction throughout the body and helps to reduce catalase activity in stressed tissues. Vitamin E is a good antioxidant vitamin and it plays an important role in the prevention of free-radical mediated damages by directly scavenging them and it helps maintain antioxidant status and in detoxification system.

CONCLUSION

The results of this study suggest that walnut oil could be effective as an antioxidant in ameliorating the toxic effects of cadmium.

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