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Antioxidant Activity of Corchorus Olitorius and Its Effect on Lipid Peroxidation in Mice

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ABSTRACT

The study examined the antioxidant and anti-lipid peroxidative activity of *Corchorus olitorius*. Antioxidant activity was examined in-vitro by 1,1,-Diphenyl-2-picrylhydrazyl radical scavenging method. Lipid peroxidation, examined by measuring malondialdehyde (MDA) levels in erythrocytes of three groups of mice fed different levels of *C. olitorius*, showed reduction in MDA from 1.38 to 0.94 µmol/g Hb and 1.22 to 0.83 µmol/g Hb in male and female mice respectively. No significant change in weights ($p>0.05$) were found in the liver, kidney, heart, spleen and lungs of the mice. Changes in body weights were significant ($p<0.05$) suggesting *C. olitorius'* potential use in obesity management.

Introduction

Antioxidants are substances found naturally in the body and in plants [1], which protect cell membranes from free-radical mediated oxidative damage [2]. Free radicals, including oxidizing compounds, are generated endogenously during mitochondrial electron transport, prostaglandin synthesis, endoplasmic reticulum oxidation, enzymatic activity and oxyhaemoglobin auto-oxidation. They may also be generated exogenously in response to metabolism of drugs, ionizing radiation, tobacco smoke, oxidizing pollutants, pesticides, and organic solvents [3,4]. In the body, free radicals alter the function and structure of the cell membrane by causing oxidative injury to polyunsaturated fatty acids, a process termed as lipid peroxidation [5]. Lipid peroxidation has been implicated in diverse pathological conditions, including arteriosclerosis, aging and cancer [6] due to the buildup of lipid peroxides such as lipid peroxyl, lipid hydroperoxide and malondialdehyde (MDA) in body tissues. The amount of MDA formed by tissue homogenates is a reliable indicator of tissue damage caused by membrane lipid peroxidation [7]. The human body boasts of its own antioxidants, e.g. glutathione peroxidase, superoxide dismutase and catalase, which protect it from lipid peroxidation. However, available evidence indicates that, these endogenous antioxidant defenses are inadequate, hence, there is the need to take in natural antioxidants from dietary sources to ensure utmost protection [1,8].

Corchorus olitorius (Linn) is a widespread leafy vegetable found in tropical Africa, Egypt, Malaysia, Philippines, South America, and the Caribbean [9]. The young leaves of the plant serve as the main source for dietary protein and minerals in several tropical countries [10]. *C. olitorius* has lately been reported to contain primary antioxidants such as 5-caffeoylquinic acid (chlorogenic acid), 3,5-dicaffeoylquinic acid, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-(6-malonylglucoside), and quercetin 3-(6-malonylgalactoside) [11]. What remains unknown, however, is the antioxidant and anti-lipid peroxidative activity of the plant. Therefore this study sought to investigate the antioxidant activity of the fresh leaves of *C. olitorius* using DPPH free-radical system and their effect on lipid peroxidation in mice.

Materials and Methods

(1) Chemicals and Reagents

1,1-Didhenyl-2-picryl hydrazyl (DPPH), thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropanane (TEP), methanol, ethanol, trichloroacetic acid (TCA), n-butanol, sodium hydroxide (NaOH), hydrochloric acid (HCl) and Butylated hydroxyl toluene (BHT) were obtained from Fluka Chemie, Switzerland. Sodium phosphate-monobasic (NaH2PO4), sodium phosphate-dibasic (Na2HPO4), diethyl ether and sodium chloride (NaCl) were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A).

(II) Experimental Animals

Seven (7) weeks old specified pathogen-free Institute Cancer Research (ICR) strain mice weighing about 20g were obtained from the Animal Experimentation Department of the Noguchi Memorial Institute for Medical Research (NMIMR) of the University of Ghana, Legon. The animals were transferred from the Barrier Facility to the Experimentation Facility and were kept for 4 days for acclimatization and observation before being used for the experiments. They were fed on pelleted animal feed obtained from Agro-Food Company Ltd (GAFCO), Tema, Ghana.

(III) Plant Material and Preparation

Fresh *C. olitorius* leaves were acquired from Madina Market, Accra (Ghana), between the 3rd and 4th of July, 2016. Two hundred and fifty grams (250 g) were washed, chopped into pieces using a blender (SANYO, SM-G300, Japan) at a medium speed and boiled in 400 ml of water and left to stand

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overnight at room temperature. The extract was separated from the crude materials using a centrifuge (SAKUMA, 300S-1, India) at a speed of 3000 rpm for 5 minutes, filtered and then freeze-dried using a freeze dryer (Heto, LL3000, UK) to yield solid materials. The freeze-dried product was stored at 4 ºC. Ethanol extract was also prepared by immersing chopped fresh *C. olitorius* leaves weighing 250 g into 500 ml of 50% ethanol solution and leaving it overnight. The extract was separated from the crude materials by centrifuging at a speed of 3000 rpm for 5 minutes. The product obtained was filtered, rotary evaporated (BUCHI, R-250 V800, Switzerland) at 60 ºC and freeze-dried as described above. This freeze-dried product was also stored at 4ºC.

(IV) Determination of Dosage for Animal Model

A consumption survey involving ten randomly selected *C. olitorius* leaf traders and consumers each was conducted at Madina Market (Ghana). Information obtained showed that, averagely 90 g of the leaves was consumed by an adult consumer per meal/day. This quantum of leaves yielded 3.14 g of freeze-dried extract after boiling in water and centrifuging. The normal daily dose of the extract for an ICR mouse was estimated as 0.91 g using Equation (1).

$$D = (M \times E) / W \quad (1)$$

Where D is the dosage of the extract (g), M is the average weight of the mouse (20 g), E is the weight of freeze-dried extract from 90 g of leaves (3.19 g) and W is the average weight of an adult consumer (70 kg) [12].

(V) Administration of Plant Extract

Three different concentrations of the aqueous plant extract were each re-suspended in 0.4 ml of distilled water and administered to three groups of ICR mice (test groups) by oral gavage, each morning for twenty-one consecutive days. The concentrations of freeze-dried extract administered were 45.57 mg/kg body weight (bwt) /day (Low group), 455.7 mg/kg bwt /day (Medium group), and 2,278.5 mg/kg bwt /day (High group). A fourth group, which served as a control was given, distilled water by oral gavage for the same period. Each group made up of six mice consisted of three males and three females. Throughout the exercise, the animals were fed on their normal diet ad libitum. Each mouse received a daily dose between 8.00 am and 9.30 am every day. The extracts administered were freshly prepared each day.

(VI)1,1,-Diphenyl-2-picrylhydrazyl (DPPH) Radical Assay

DPPH radical scavenging activity was determined according to the method of Lee et al. (2003) [13] quoting Brad-Williams et al. [14], with slight modification in this experiment. Briefly, 1.25 mM DPPH was prepared in methanol. Fifty microlitres (50 µl) of 2 mg/ml butylated hydroxyl toluene (BHT), 25 mg/ml ethanol or aqueous extract of *C. olitorius* were separately added to 2.95 ml of the DPPH solutions and the decrease in absorbance for each sample at 517 nm was measured using a spectrophotometer (Shimadzu, UV-120-02, USA) at regular interval of 1 minute for 20 minutes. The control solution consists of 50 µl of distilled water added to 2.95 ml of DPPH solution.

(VII) Sampling of Blood from Mice

Blood was collected from the tail vein after a tail snip prior to extract administration. On day 21, the animals were anesthetized with diethyl ether and blood was again collected by cardiac puncture into tubes containing heparin.

(VIII) Erythrocytes Lipid Peroxidation Assay

Blood samples were centrifuged at 3,000 rpm for 15 minutes to remove the plasma and buffy coat (consisting of leukocytes and platelets). The erythrocytes were washed three times in phosphate buffered saline, pH 7.4. The packed cells were suspended in equal volume of the buffered saline. The amount of MDA in the erythrocyte suspension was measured according to method of Ledwozyw et al. [15]. Briefly, MDA was estimated by the thiobarbituric acid reactions as follows: 0.5 ml of erythrocyte suspensions were mixed with 2.5 ml of 1.22 mM TCA in 0.6 mM HCl and allowed to stand for 15 minutes. A volume of 1.5 mm of TBA solution was added and thereafter heated for 30 minutes in a boiling water bath. The mixtures were cooled to room temperature and 2.0 ml of n-butanol was added, vortexed for 3 minutes and centrifuged at 1,500 rpm for 10 minutes. The organic layers were removed and their absorbance measured at 532 nm against n-butanol as blank. The concentration of MDA in the samples was determined from a standard curve plotted by using free MDA prepared from TEP 1, 1, 3, 3-tetrathoxypropane. Finally, the concentration of MDA was expressed as µmol/g heamoglobin.

(IX) Body and Organ Weight

Body weights of animals were recorded at day 0 and every week thereafter, after overnight fasting for 3 weeks. At termination, the animals were then euthanized by cervical dislocation and organ (liver, kidneys, spleen, heart, and lungs) weights were recorded for all animals. Paired organs were weighed as a unit.

(X) Statistical Analysis

Values were expressed as mean ± standard error of the mean (SEM). Comparisons between treatment means were performed using analysis of variance (ANOVA) was performed using Statistical Package for Social Scientist (SPSS version 20.0) to determine statistical significance. The 0.05 level of probability was used as the criteria of significance in all instances.

Results and Discussion

(I) Free Radical Scavenging Activity of *C. olitorius* and BHT

Reduction of DPPH, a stable free radical, is one of the commonest methods used in antioxidant assay. DPPH gives a strong maximum absorption at 517 nm because of its odd electron. As the odd electron of this radical becomes paired off in the presence of a hydrogen donor, that is, a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolourization is stoichiometric with respect to the number of electrons captured. This reaction has been widely used to test the ability of compounds to act as free-radical scavengers and to evaluate the antioxidant activity of foods and plant extracts [4]. In this study, BHT showed a relatively faster free radical scavenging action than both aqueous and ethanol extracts of *C. olitorius* over the entire reaction time (Fig. 1). Whilst the change in absorbance of DPPH radical induced by BHT ranged from 0.41 ± 0.01 to 0.20 ± 0.01, that of the ethanol and aqueous extracts ranged from 0.43 ± 0.00 to 0.37 ± 0.01 and 0.43 ± 0.00 to 0.33 ± 0.07, respectively. This was expected since BHT is a pure compound unlike the aqueous and ethanol extracts of *C. olitorius*, which had other compounds present in addition to the antioxidants, thus exhibiting mild inhibitory activity against the DPPH radicals. These non-antioxidant compounds may also have accounted for the initial absorbance interruptions observed in the extracts-DPPH assay. The mild scavenging activity exhibited by the aqueous and the ethanol extracts can also be explained in terms of the sensitivity of the DPPH assay.

Earlier work done by Lee et al. [13], showed that cocoa, black tea, red tea and green tea exhibited more antioxidant activity with 2,2´-azino-bis(3-ethylbenzthiaz oline-6-sulfonic acid) (ABTS) radicals than DPPH radicals. The differences were attributed to absorbance interruption at 517 nm by other compounds in the DPPH assay. Comparing the free radical scavenging ability of the two extracts, the aqueous

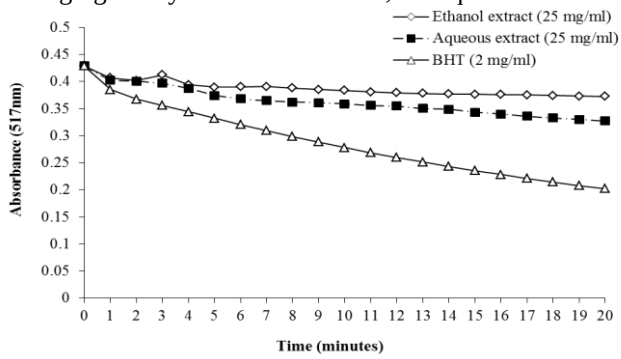

Figure 1. Time course of absorbance reduction of DPPH by aqueous and ethanolic leave extract of *Corchorus olitorius* and butylated hydroxytoluene (BHT).

showed a stronger effect on DPPH than the ethanol extract. This was unexpected because earlier work done by Azuma et al. [11] identified six strong phenolic antioxidants [5-caffeoylquinic acid (chlorogenic acid), 3,5-dicaffeoylquinic acid, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-(6-malonylglucoside), and quercetin 3-(6-malonylgalacto side] found abundantly in the *C. olitorius* leaves. These compounds are either insoluble or poorly soluble in water but highly soluble in organic solvents such as ethanol. The findings of this study suggest that, *C. olitorius* also contains water soluble substances which may have very strong antioxidant properties.

(II)Malondialdehyde (MDA) Levels in Erythrocyte Lysates

There were significant differences ($p<0.05$) in the mean values of MDA, a by-product of lipid peroxidation, among the treatment groups of both sexes of the mice (Fig. 2 and 3). Compared to the control groups, the extract treated groups generally showed a dose-dependent decrease in MDA levels. However, the male animals treated with 455.7 mg/kg bwt and 2,278.5 mg/kg bwt of the extract showed no significant difference ($p>0.05$) in their MDA levels and also, the MDA level of female animals treated with 45.57 mg/kg bwt of the extract were not significantly different from the control groups ($p>0.05$). These findings may be as result of multi-concentration defense mechanisms against free radical-induced lipid peroxidation which are found in erythrocytes [16].

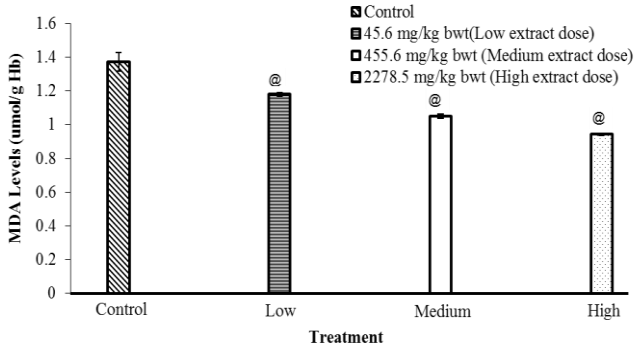

Figure 2. Malondialdehyde content of erythrocytes lysates in male mice after treatment with *C. olitorius* leaf extract.

Catalase and superoxide dismutase are widely distributed in all animal tissues and they plays a profound role in protecting erythrocytes against oxidative stress [17]. A high concentration of vitamin E and reduced glutathione in red blood cells also provide a major

@ Value significantly different from control, $p<0.05$

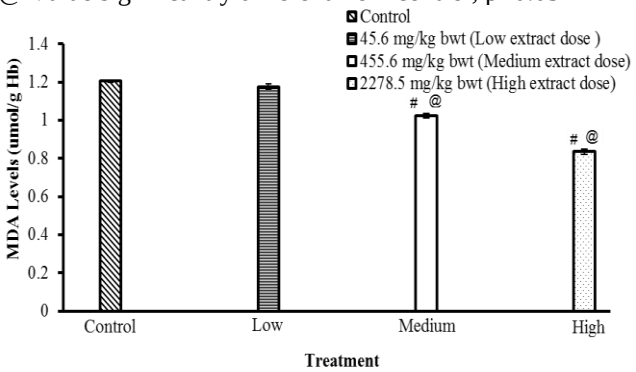

Figure 3. Malondialdehyde content of erythrocytes lysates in female mice after treatment with *C. olitorius* leaf extract.

@ Value significantly different from control, $p<0.05$

Value significantly different from low dose group, $p<0.05$ defense mechanism against free radical-induced lipid peroxidation [18,19]. Nonetheless, the presence of MDA in the erythrocytes reflects insufficient antioxidant potential in the erythrocytes of the mice.

Generally, the MDA levels of the female animals were lower than that of the males. MDA levels in the males dropped from 1.38 ± 0.06 µmol/g Hb to 0.94 ± 0.01 µmol/g Hb whilst that of the females dropped from 1.22 ± 0.05 µmol/g Hb to 0.83 ± 0.02 µmol/g Hb. This finding is consistent with results from studies carried by Borras et al. [20] and Vina et al. [21]. The authors found that female Wistar rats had decreased reactive oxygen species (ROS) production, higher antioxidant defenses and lower oxidative macro-molecule damage in comparison to the males. According to Kofler et al. [22], estrogen found in females interferes with the free radical cascade by blocking Cytochrome c release from the mitochondria leading to the decrease in the formation of superoxide anions. It therefore suggests that, because the female mice already have benefits of antioxidant effects of endogenous estrogen, less lipid peroxidation occurred in them as compared to the males.

(III) Body Weight Changes

There were significant differences ($p<0.05$) in the percent change in body weight among the different levels of treatment of both animal sexes (Fig. 4 and 5). Also, the difference in the mean values of the percent change in body weight among the different weeks was significantly greater ($p<0.05$) than would be expected by chance after allowing for

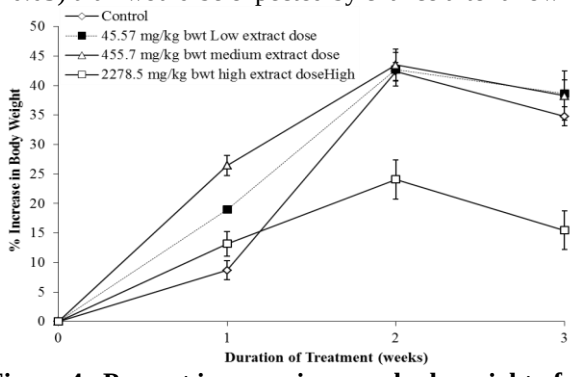

Figure 4. Percent increase in mean body weight of male mice given *C. olitorius.*

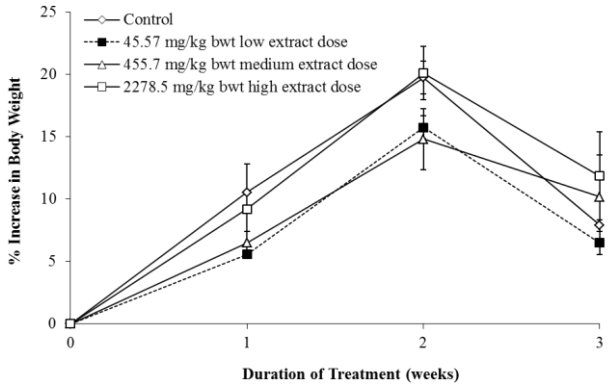

Figure 5. Percent increase in mean body weight of female mice given *C. olitorius.*

effects of differences in treatment. All the treatment groups and the controls gained weight over the first two weeks with no clear dose-dependent effects in the body weight. However, weight gained in high dose (2,278.5mg/kg) male group was significantly lower. Antioxidants are considered to play vital role in preventing oxidative stress through reducing NADPH oxidase expression, accounting for the anti-obesity effect [23, 24]. Studies have also shown that quercetin derivatives inhibit ROS production and improve lipid metabolism by enhancing β-oxidation. Increased β-oxidation enhances energy expenditure thus helping to prevent obesity [25]. These scientific facts could best explain the suppression of weight gain observed in the male mice. The reason for the sudden decline in body weight of all the mice after the second week is not certain. The weight loss could be caused by several factors which include excessive running due to environmental disturbance, changes in nutritional composition of the feeds, contamination of the drinking water, change in macro- and micro-environmental conditions, infection and metabolic processes. The fact that the weight loss also occurred in the controls has ruled out the treatment as a possible cause.

(IV) Changes in Organ Weight

Post mortem examinations on the internal organs indicated no significant differences ($p>0.05$) in the weights of the liver, kidney, heart, lung and the spleen of both the treated animals and the controls (Tables 1 and 2). This observation agrees with those made by Al-Orf [26] who after administering oxidized phosphatidylcholine to rats observed no significant difference in the weights of the thymus, spleen, heart, kidney, liver and lungs of both treated and control. However, he observed that the MDA levels in the organs significantly increased in treated rats as compared to the unoxidized control. This seems to suggest that, organ weight could not be an appropriate indicator of the existence of oxidative stress. Notwithstanding, other studies have reported weight loss in

Table 1. Terminal wet weights of organs isolated from male mice given various doses of *C. olitorius* orally for three (3) weeks.

Organ	Organ weights (% body weight)			
	Control	Treatment groups (mg/kg)		
		45.57 (Low)	455.7 (Medium)	2278.5 (High)
Liver	4.11±0.07	4.25±0.12	4.51±0.16	4.02±0.14
Kidney	1.67±0.12	1.43±0.06	1.65±0.08	1.41±0.01
Heart	0.60±0.07	0.51±0.02	0.52±0.03	0.59±0.09
Lungs	0.70±0.13	0.73±0.03	0.75±0.02	0.74±0.06
Spleen	0.26±0.02	0.23±0.03	0.32±0.02	0.26±0.02

Results are means ± SEM. n = 3

Table 2. Terminal wet weights of organs isolated from female mice given various doses of *C. olitorius* orally for three (3) weeks.

Organ	Organ weights (% body weight)			
	Control	Treatment groups (mg/kg)		
		45.57 (Low)	455.7 (Medium)	2278.5 (High)
Liver	4.27±0.56	4.04±0.19	4.09±0.27	4.44±0.14
Kidney	1.29±0.17	1.29±0.05	1.11±0.07	1.22±0.06
Heart	0.59±0.00	0.52±0.03	0.43±0.08	0.43±0.01
Lungs	0.85±0.07	1.01±0.01	0.87±0.05	0.98±0.09
Spleen	0.24±0.05	0.42±0.08	0.35±0.09	0.34±0.02

Results are means ± SEM. n = 3

liver [27, 28], lung, heart, spleen and kidneys [28] following the administration of dietary antioxidants.

Conclusion

The DPPH assay has demonstrated that *C. olitorius* has significant antioxidant properties. The study also indicates that aside the phenolic antioxidants which were earlier identified, *C. olitorius* also contain potent water-soluble antioxidants and these have provided protection against lipid peroxidation in mice. Further findings indicate that the intake of large quantities of this vegetable could reduce weight gain in males. This could be of significance in the management of human patients who are obese or have high body mass index, a risk factor for diabetes and hypertension with/or cardiovascular disorder complications.

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