Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: Florence O. Jimoh, Seyi A. Babalola & Musa T. Yakubu (2009) Assessment of the antioxidant potential of *Cnidoscolous chayamansa*, Pharmaceutical Biology, 47:9, 903-909, DOI: 10.1080/13880200902942444

To link to this article: https://doi.org/10.1080/13880200902942444

Published online: 27 Aug 2009.

Pharmaceutical Biology, 2009; 47(9): 903–909

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RESEARCH ARTICLE

Assessment of the antioxidant potential of *Cnidoscolous chayamansa*

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Abstract

The current study is an effort to investigate the chemical constituents and antioxidant potential of *Cnidoscolous chayamansa* McVaugh (Euphorbiacea) root on carbon tetrachloride-induced liver damage. Albino rats were grouped into four: A - D. Groups A and B received 1 mL/kg BW of olive oil and 1 mL/kg body weight (BW) of carbon tetrachloride (CCl_4), respectively, for 8 days while those in C and D received 1 mL/ kg BW each of CCl_4 and 500 and 1000 mg/kg BW of aqueous extract of *C. chayamansa* root, respectively, for the same period. Chemical analysis of the plant root revealed the presence of tannins, phenolics, flavonoids, saponins, Fe, Zn, Mg, Ca, vitamins A and C. Administration of CCl_4 resulted in significant increase ($P < 0.05$) in liver malondialdehyde concentration while the activities of liver alkaline phosphatase, superoxide dismutase, glutathione peroxidase and catalase were significantly reduced ($P < 0.05$). Simultaneous administration of CCl_4 and the plant extract at 500 and 1000 mg/kg BW produced values of these biochemical parameters that compared favourably with the control ($P > 0.05$) in addition to increasing superoxide dismutase activity in the liver ($P < 0.05$). We conclude that aqueous extract of *C. chayamansa* root possessed antioxidant activity and protected the hepatocyte against CCl_4-induced damage. The antioxidant activity of the plant may be due to its chemical constituents.

Keywords: *Antioxidant; carbon tetrachloride; Cnidoscolous chayamansa; Euphorbiacea; hepatocyte; liver damage; simultaneous administration*

Introduction

The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against hepatic damage. An expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole, or their identified ingredients with antioxidant properties have substantial protective effects against free radical associated diseases (Tsao et al., 2004). For instance, the phenolic content of the chloroformic extract of *Eucalyptus maculata* Hook (Myrtaceae) have been shown to exhibit hepatoprotective and antioxidant properties (Mohammed et al., 2005).

Cnidoscolous chayamansa, McVaughn (Euphorbiacea), commonly called "Chaya", is an attractive shrub of 3-5 m tall which grows in the northern part of Nigeria. The white flowers, which are usually borne on cyme-branched inflorescences, may contain 3-forked arrangements in which the pistillate flowers are located on the basal fork. The staminate flowers are expanded distally from the base of the lobes. It has potential uses as a leafy vegetable and/or as a medicinal plant (Kuti & Torres, 1996). Traditionally, the shoots and leaves of *C. chayamansa* have been recommended as a laxative, diuretic, circulation stimulant, to improve digestion, to stimulate lactation, and to harden fingernails. The aqueous extract (as used in folklore medicine) of the stem, leaves and roots have been used in the management of a number of ailments such as diabetes, obesity, kidney stones, hemorrhoids, acne, and eye problems (Rowe, 1994). The entire plant, including the root, can be dried and ground for use as animal feed.

Several studies (Recknagel & Glende, 1973; Anand et al., 1992; Akanji et al., 2004) have shown that carbon

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(Received 01 April 2008; revised 21 May 2008; accepted 27 June 2008)

ISSN 1388-0209 print/ISSN 1744-5116 online © 2009 Informa UK Ltd
DOI: 10.1080/13880200902942444

tetrachloride (CCl_4) is one of the agents that injure hepatocytes and its organelles by peroxidation of the membrane lipid and denaturation of proteins. Recknagel et al. (1989) reported that the oxidative damage through free radical generation is among the various mechanisms involved in the hepatotoxic effects of CCl_4. CCl_4 intake is followed by spontaneous or enzymic homolytic split, leading to the formation of trichloromethyl radical, which could modify molecules, probably lipids, leading to peroxidation of polyunsaturated fatty acids in the cell membrane (Masuda & Nakamura, 1990). This may result in its fragmentation with loss of both lipid and proteins or probably conversion of other cellular molecules to secondary free radicals that extend the injury (Recknagel & Glende, 1973). It has been shown that CCl_4-induced lipid peroxidation can be obstructed by natural antioxidants (Subramanian et al., 1999; Wang et al., 2000). The screening of medicinal plants with inhibitory potentials against peroxidation of lipids and subsequent free radicals can thus be exploited as a phytotherapeutic approach to disease prevention.

While the nutritional value of chaya has been demonstrated (Booth et al., 1992), none of the purported therapeutic values of chaya has been substantiated with scientific experimentation. In a previous work, antioxidant potential of the leaf of *C. chayamansa* was established (Kuti & Konuru, 2004), but information remains scanty on the antioxidant potentials of other parts of the plant. Therefore, this study explored the antioxidant potential of aqueous extract of *C. chayamansa* root in CCl_4-induced rat liver damage.

Materials and methods

Plant material

The plant sample obtained within the premises of the main campus of the University of Ilorin, Ilorin, Nigeria between August and September, 2005 was authenticated by Felix Usang of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen was deposited in the herbarium unit of FRIN under a voucher number, FHI 107627.

Animals

Adult albino rats (*Rattus norvegicus*) weighing between 180-200 g were obtained from the animal holding unit of the Department of Biochemistry, Faculty of Science, University of Ilorin. The animals were kept in well-ventilated house conditions (temperature 28°–31°C; photoperiod 12 h natural light and 12 h dark; humidity 50–55%) with free access to rat pellets (Bendel Feeds and Flour Mills, Ewu, Nigeria) and tap water. This study was conducted in accordance with the ethical norms approved by the Ethical Committee on the Care and Use of Experimental Animals of the Department of Biochemistry, University of Ilorin.

Assay kits and other reagents

The assay kits for catalase, superoxide dismutase, glutathione peroxidase and alkaline phosphatase were obtained from Randox Laboratories Ltd., Crumlin, County Antrim, UK. Co-Atrim, London. Carbon tetrachloride was a product of Reeve Angel Scientific, Balham, London. All other reagents used were of analytical grade and were prepared in all glass distilled water except otherwise specified.

Preparation of extract

The procedure described by Yakubu (2006) was used. Briefly, the roots of *C. chayamansa* were washed in tap water, cut into small pieces and then oven-dried at 40°C for 48 h to constant weight. The dried pieces were then pulverized with an electric blender (Blender/Miller III, model MS–223, Taipei, Taiwan) and the resulting powder was stocked in a plastic container. The powder (200 g) was weighed and boiled in 2 L of distilled water for 30 min and thereafter allowed to cool at room temperature. This was later filtered with Whatman No. 1 filter paper (Maidstone, UK) and the resulting filtrate concentrated on a steam bath to give 13.37 g of brownish black residue which is equivalent to a yield of 6.69%.

Determination of chemical constituents

Phytochemical screening of *C. chayamansa* root was carried out according to the method described by Odebiyi and Sofowora (1978) for the detection of tannins, phenolics, alkaloids, steroids, phlobatannins, glycosides, and flavonoids. The quantitative determination was done according to standard procedures as described by El-Olemy et al. (1994). The method described by Indrayan et al. (2005) was used for the determination of mineral elements while that described by Blanco et al. (1994) was used for the determination of vitamins A and C.

Preparation of CCl_4

Carbon tetrachloride (30 % ,v/v) was suspended in olive oil; 1 mL/kg BW dosage of the preparation was administered intraperitoneally to the rats (Bhandarkar & Khan, 2004; Shahjahan et al., 2004).

Animal grouping and treatment

Sixty albino rats were allowed to acclimatize for 7 days before the start of administration. The animals were

completely randomized into four groups and treated as follows:

A. treated intraperitoneally with 1 mL/kg BW of olive oil
B. treated intraperitoneally with 1 mL/kg BW of CCl_4
C. treated intraperitoneally with 1 mL/kg BW of CCl_4 followed by oral administration of 500 mg/kg BW of the plant extract
D. treated intraperitoneally with 1 ml/kg BW of CCl_4 followed by oral administration of 1000 mg/kg BW of the plant extract

The treatments were done at 24 h intervals for eight days. The CCl_4 and the extracts were administered simultaneously. Five rats each from the groups were sacrificed 24 h after 2, 4, and 8 daily doses (Akanji et al., 2004).

Preparation of liver homogenate

The method described by Yakubu (2006) was used for the preparation of liver homogenate. Briefly, under light ether anesthesia, the animals were quickly dissected and the liver was excised from the animals. The organ was cleaned of superficial fatty layer, weighed and later transferred into 0.25 M sucrose solution. The liver was later blotted with tissue paper, cut very thinly with sterile scalpel blade and homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were further centrifuged at 1340 × *g* for 15 min to obtain the supernatant, which was then aspirated with Pasteur pipette into sample bottle, stored overnight at 4°C before being used for the biochemical assays.

Determination of biochemical parameters

The protein concentration in the liver homogenates was determined by the biuret method of Gornall et al. (1949) while lipid peroxidation was determined spectrophotometrically as described by Varhney and Kale (1990). The activities of alkaline phosphatase (EC. 3.1.3.1), superoxide dismutase (EC. 1.15.1.1), catalase (EC. 1.11.1.6) and glutathione peroxidase (EC. 1.11.1.9) were determined by the methods described by Wright et al. (1972), Roos et al. (1959), Beers and Sizer (1952), and Rotruck et al. (1973), respectively.

Statistical analysis

The determinations were replicated five times. Results were expressed as the mean ± SD. Means were analyzed using a one-way analysis of variance, followed by the Duncan Multiple Range Test to determine significant differences in all the parameters. Differences with values of $P < 0.05$ were considered statistically significant (Mahajan, 1997).

Results

Phytochemical screening of aqueous extract of *C. chayamansa* root revealed the presence of flavonoids, phenolics, tannins, and saponins while alkaloids, glycosides, steroids, and phlobatannins were not detected (Table 1). Saponins however had the highest percentage concentration (0.69%), followed by phenolics (0.35%) and tannins (0.10%) while flavonoid was present in a trace amount (0.06%). The plant root also has vitamins A and C, with vitamin C almost three times more than

Table 1. Chemical constituents of *Cnidoscolous chayamansa* root.

Phytochemicals	Concentration
Alkaloids (%)	Not detected
Tannins (%)	0.10 ± 0.01
Phenolics (%)	0.35 ± 0.02
Glycosides (%)	Not detected
Flavonoids (%)	0.06 ± 0.001
Steroids (%)	Not detected
Phlobatannins (%)	Not detected
Saponins (%)	0.69 ± 0.04
Vitamin A (mg/100g)	0.59 ± 0.02
Vitamin C (mg/100g)	1.70 ± 0.05
Iron (ppm)	96.00 ± 0.41
Zinc (ppm)	3.50 ± 0.01
Magnesium (ppm)	456.50 ± 0.41
Calcium (ppm)	275.50 ± 0.22

Values are mean of three replicates ± SD.

Table 2. Effect of administration of CCl_4 and aqueous extract of *C. chayamansa* roots on the alkaline phosphatase activities (UI) of rat liver.

	Days after administration		
Groups	2	4	8
Olive oil	128.09 ± 5.68[a]	130.31 ± 4.27[a]	129.10 ± 4.21[a]
CCl_4 only	108.55 ± 6.38[b]	88.30 ± 4.01[b]	52.82 ± 3.80[b]
CCl_4 + 500 mg/kg	140.45 ± 4.43[c]	131.88 ± 4.10[a]	130.40 ± 5.22[a]
BW of extract	(29.39%)	(49.35%)	(146.88%)
CCl_4 + 1000 mg/kg	126.72 ± 9.33[a]	129.86 ± 4.83[a]	130.93 ± 4.01[a]
BW of extract	(16.74%)	(47.07%)	(147.88%)

n = 5 ± SD; [a-c]Test values carrying superscripts different from the control group (olive oil only) for each day are significantly different ($P < 0.05$); values in parentheses indicate percentage recovery compared to CCl_4.

Table 3. Effect of administration of CCl_4 and aqueous extract of *C. chayamansa* roots on the rat liver malondialdehyde concentration (nmoles of MDA formed/mg protein/h).

	Days after administration		
Groups	2	4	8
Olive oil	4.12 ± 0.19^{a}	4.13 ± 0.20^{a}	4.11 ± 0.21^{a}
CCl_4 only	6.01 ± 0.17^{b}	8.26 ± 0.06^{d}	11.25 ± 0.58^{b}
CCl_4 + 500 mg/kg BW of extract	5.86 ± 0.33^{c}	4.26 ± 0.05^{a}	4.12 ± 0.18^{a}
CCl_4 + 1000 mg/kg BW of extract	4.12 ± 0.22^{a}	4.14 ± 0.17^{a}	4.13 ± 0.19^{a}

n = 5 ± SD; [a-d]Test values carrying superscripts different from the control group (olive oil only) for each day are significantly different ($P < 0.05$).

Table 4. Effect of administration of CCl_4 and aqueous extract of *C. chayamansa* roots on the rat liver superoxide dismutase (unit of activities/mg protein).

	Days after administration		
Groups	2	4	8
Olive oil	19.74 ± 0.56^{a}	19.79 ± 0.48^{a}	19.60 ± 0.71^{a}
CCl_4 only	13.90 ± 0.34^{b}	14.15 ± 0.82^{b}	12.59 ± 0.71^{b}
CCl_4 + 500 mg/kg BW of extract	20.73 ± 0.10^{a} (49.14%)	20.78 ± 0.07^{a} (46.86%)	23.17 ± 0.02^{c} (84.03%)
CCl_4 + 1000 mg/kg BW of extract	23.57 ± 0.31^{c} (69.57%)	24.53 ± 0.85^{c} (73.36%)	27.24 ± 0.51^{d} (116.36%)

n = 5 ± SD; [a-d]Test values carrying superscripts different from the control group (olive oil only) for each day are significantly different ($P < 0.05$); values in parentheses indicate percentage recovery compared to CCl_4.

Table 5. Effect of administration of CCl_4 and aqueous extract of *C. chayamansa* roots on the rat liver catalase (micromoles of H_2O_2 decomposed/mg protein/min) activities.

	Days after administration		
Groups	2	4	8
Olive oil	3.07 ± 0.42^{a}	3.09 ± 0.38^{a}	3.08 ± 0.44^{a}
CCl_4 only	1.78 ± 0.58^{b}	1.73 ± 0.10^{b}	1.49 ± 0.14^{b}
CCl_4 + 500 mg/kg BW of extract	2.47 ± 0.12^{c} (38.76%)	2.66 ± 0.16^{c} (53.76%)	3.21 ± 0.02^{c} (115.44%)
CCl_4 + 1000 mg/kg BW of extract	3.08 ± 0.07^{a} (73.03%)	3.07 ± 0.21^{a} (77.46%)	3.10 ± 0.03^{a} (108.05%)

n = 5 ± SD; [a-c]Test values carrying superscripts different from the control group (olive oil only) for each day are significantly different ($P < 0.05$); values in parentheses indicate percentage recovery compared to CCl_4.

Table 6. Effect of administration of CCl_4 and aqueous extract of *C. chayamansa* roots on the rat liver glutathione peroxidase (microgram/mg protein/) activities.

	Days after administration		
Groups	2	4	8
Olive oil	5.27 ± 0.11^{a}	5.29 ± 0.10^{a}	5.30 ± 0.07^{a}
CCl_4 only	2.09 ± 0.07^{b}	1.63 ± 0.05^{b}	1.02 ± 0.02^{b}
CCl_4 + 500 mg/kg BW of extract	2.74 ± 0.02^{c} (31.10%)	3.48 ± 0.08^{c} (113.50%)	4.30 ± 0.11^{c} (321.57%)
CCl_4 + 1000 mg/kg BW of extract	5.28 ± 0.09^{a} (152.63%)	5.30 ± 0.04^{a} (225.15%)	5.31 ± 0.02^{a} (420.59%)

n = 5 ± SD; [a-c]Test values carrying superscripts different from the control group (olive oil only) for each day are significantly different ($P < 0.05$); values in parentheses indicate percentage recovery compared to CCl_4.

vitamin A (Table 1); magnesium, calcium, iron, and zinc, with the highest being magnesium (456.5 ppm), followed by calcium (275.5 ppm), iron (96 ppm), and zinc (3.5 ppm) (Table 1).

Tables 2–6 depict the pattern of effect of administration of CCl_4 only and its combination with aqueous extract of *C. chayamansa* root on various biochemical indices of antioxidant investigated. CCl_4 administration alone resulted in significant decrease ($P < 0.05$) in

the liver alkaline phosphatase activity leading to half the control value by the end of the experimental period (Table 2). Administration of the plant extract however reversed this trend to control values. This reversal commenced from after the second dose for the simultaneous administration of CCl_4 and 500 mg/kg body weight of the extract while it was immediate with the simultaneous administration of CCl_4 and 1000 mg/kg body weight of the extract (Table 2).

Administration of CCl_4 alone significantly increased ($P<0.05$) the malondialdehyde concentration in the rat liver and by the end of the experimental period, the concentration had increased 2.7-fold (Table 3). Simultaneous administration of the plant extract at 500 and 1000 mg/kg body weight produced values that compared favourably ($P>0.05$) with the control.

CCl_4 administration alone produced significant reduction ($P<0.05$) in the activities of superoxide dismutase, catalase and glutathione peroxidase in the rat liver (Tables 4–6). While the simultaneous administration of CCl_4 and 500 mg/kg BW of the extract produced values of superoxide dismutase activity that compared favourably ($P>0.05$) with the control, the simultaneous administration of CCl_4 and 1000 mg/kg BW resulted in significant increase in the enzyme activity (Table 4). Similarly, while the activities of catalase and glutathione peroxidase were not reversed to the control values following the simultaneous administration of CCl_4 and the 500 mg/kg body weight of the extract, the simultaneous administration of CCl_4 and 1000 mg/kg body weight reversed the activities of the enzymes back to the control (Tables 5 and 6).

Discussion

The various biochemical parameters evaluated in this study are indices that can be used to assess the antioxidant potential of chemical compounds and or plant extracts. Several authors (Bhandarkar & Khan, 2004; Shahjahan et al., 2004; Dahiru et al., 2005) have used indices such as the activities of alkaline phosphatase, superoxide dismutase, glutathione peroxidase, catalase, and the level of malondialdehyde to assess the antioxidant potentials of plant extract against CCl_4-induced tissue damage in rats, hence their use in this study.

The presence of flavonoids, saponins, phenolics, vitamins A and C, iron, magnesium, and zinc in the plant root extract supports the antioxidant potentials of *C. chayamansa*. Flavonoids, saponins and phenolics are known to possess antioxidant and hepatoprotective activity (Vijyan et al., 2003). The phytochemicals may function either by acting as chain-breaker or scavengers of free radicals. Similarly, vitamin C also "scavenges" aqueous peroxyl radicals or may donate electrons to free radicals. It may work in concert with vitamin E and glutathione peroxidase to stop free radical chain reactions. Zinc protects sulfhydryl groups against oxidation and may also inhibit the production of reactive oxygen species by transition metals (Bray & Bettger, 1990). Fe, Mg and Zn are cofactors for catalase and superoxide dismutase, respectively.

The hepatotoxic effect of CCl_4 has been attributed to its metabolism by cytochrome P_{450} to yield toxic trichloromethyl radicals that act as free radical initiators (Farber & Gerson, 1984; Saravanan et al., 2003). These free radicals increase hepatic lipid peroxidation level in CCl_4 toxicity and thus pathogenesis of liver injury.

Alkaline phosphatase is an ectoenzyme of the hepatocyte's plasma membrane. It is one of the enzymes used to assess the integrity of the cells following the administration of chemical compounds (Akanji et al., 2004; Yakubu, 2006). It also plays an important role in maintaining cell membrane permeability. CCl_4-mediated acute toxicity increases permeability of the hepatocyte membrane and thus leakage of cellular components. The significant decrease in alkaline phosphatase activity following the administration of CCl_4 alone further confirms damage on the cell membrane by peroxidation of the polyunsaturated fatty acids present on the membrane and denotes damage to the hepatic cells (Singh et al., 1999; Akanji et al., 2004). The non-significant effect on the activity of the enzyme following the administration of the plant extract is an indication that the plant extract has anti-lipoperoxidative bioactive agents.

It has been shown that free radicals generated by CCl_4 damage increase hepatic lipid peroxidation through the formation of lipid peroxides like malondialdehyde (MDA) (Farber & Gerson, 1984; Recknagel et al., 1989). Therefore, the significant increase in the liver MDA level following the administration of CCl_4 may be attributed to enhanced lipid peroxidation, leading to hepatic damage. This may also be due to failure of endogenous antioxidant defense mechanisms to prevent the formation of excessive free radicals. This is in agreement with the findings of Kalpowitz et al. (1986). However, in the present investigation, it is observed that the aqueous extract of *C. chayamansa* roots enhanced the reversal of the increased levels of MDA back to the control values. It is possible that the plant extract has prevented the formation of hepatotoxic free radicals by interfering with cytochrome P_{450} (Nadeem et al., 1997) or might have promoted its glucuronidation (Gilman et al., 1992). The ability of the plant extract to ameliorate the increased levels of MDA might be attributed to interplay of chemical components present in the plant root which are promoters of antioxidant activity. Saponins

have been implicated to exhibit antioxidant activity by reducing the levels of lipid hydroperoxides (Rodrigues et al., 2005). The saponins present in the plant extract may thus be responsible for the reduction in the level of MDA.

Superoxide dismutase (SOD) is one of the chief cellular defense enzymes that dismutate superoxide radical to H_2O_2 and oxygen. The reduction in the activities of SOD observed in this study following the administration of CCl_4 which suggests oxidative stress agrees with the report of Ohla et al. (1995). This effect was, however, ameliorated by the aqueous plant extract. The increase in SOD activities observed in this study may be attributed to the presence of cofactors of zinc and iron which are needed not only for the proper functioning of the enzyme but for its synthesis. The high levels of SOD may therefore protect the cell against reactive oxygen species-induced alteration of macromolecules. It may also complement the antioxidant defense mechanism of the animals (Fridovich, 1975). The plant extract has also enhanced the adaptive nature of the rat system against the effects of the free trichloromethyl radical.

Catalases which are heme-containing proteins protect the cells from toxic effects of reactive oxygen species by converting hydrogen peroxide to water and molecular oxygen. The reduction in the activity of catalase by CCl_4 administration is an indication of damage to the hepatic cells. This agrees with the report of Singh et al. (1999). The ability of the plant extract to revert the reduced catalase activity further buttresses the antioxidant and antihepatotoxic potentials of the plant extract.

Glutathione peroxidase constitutes the first line of defense against free radicals (Bhandarkar & Khan, 2004). It reduces H_2O_2 to H_2O by oxidizing glutathione (GSH) which requires trace metal cofactors like copper, zinc or manganese for maximal efficiency. In this study, the significant increase in the activity of glutathione peroxidase following the administration of the plant extract may be adduced to the presence of elements such as zinc that might have enhanced the synthesis of the enzyme.

From the present study, it can be concluded that the aqueous extract of *Cnidoscolous chayamansa* root especially at the dose of 1000 mg/kg BW could protect against CCl_4-induced liver damage in rats. These hepatoprotective and anti-lipoperoxidative potentials of the plant extract may be due to activation of antioxidant systems made possible by the chemical constituents of the plant root.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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