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Protective Effects of Some Tropical Vegetables against CCl_4 Induced Hepatic Damage

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Abstract: In the present study, ethanolic extracts of some tropical vegetables were investigated for their hepatoprotective effect against CCl_4 induced liver damage in rats. Carbon tetrachloride at a dose of 0.5 mL kg^{-1} body weight (b.w) produced liver damage in rats as manifested by the rise in the levels of ALT (IU L^{-1}), AST (IU L^{-1}) and total protein (g L^{-1}) in the serum (40.60±3.50, 80.60±5.10, 73.20±1.87) and in the liver homogenate (1300.00±7.38, 1660.00±13.69, 250.00±7.51) compared to the control. The extracts at a dose of 250 and 500 mg kg^{-1} bw were administered to the CCl_4 treated rats. The vegetables produced a significant hepatoprotective effect by decreasing the serum levels of ALT, AST and total protein in the range of 11.21±1.90-16.22±1.00, 29.00±2.70-48.00±2.10, 62.10±2.40-70.13±2.00 at a dose of 250 mg kg^{-1} and 13.00±1.20-21.00±1.30, 40.00.±2.5-59.00±2.20, 68.00±2.40-72.00±2.10 at a dose of 500 mg kg^{-1}. Similar results were obtained for liver homogenate levels of ALT, AST and total protein with a decreasing values compared to the control; 900.00±3.05-1020.00±4.25, 1150.00±5.57-1530.00±4.99, 150.00±3.12-185.00±3.00 and 900.00±3.05-1030.00±8.80, 1400.00±6.95-1530.00±8.50, 165.00±5.50-210.00±4.41 at a dose of 250 and 500 mg kg^{-1} b.w respectively. Furthermore, the effect of the extracts on lipid peroxidation, measured as Malondialdehyde (MDA) was estimated on the liver homogenate. A significant hepatoprotective effect was also noticed with a decreased value of the MDA levels: 46.00±0.08-52.00±0.06 and 47.00±0.07-60.00±0.10 at a dose of 250 and 500 mg kg^{-1} bw respectively. It can be concluded that all the evaluated vegetables exhibit good hepatoprotective activities, though at varying degrees.

Key words: Vegetables, hepatoprotective, carbon-tetrachloride, lipid peroxidation

Introduction

The importance of active oxygen and free radicals as exacerbating factors in cellular injury and the aging process has attracted increasing attention over the years. Carbon tetrachloride (CCl_4) is a potent hepatoxin producing centrilobular hepatic necrosis, which causes liver injury. CCl_4 induced liver injury depends on a toxic agent that has to be metabolized by the liver NAPDH-Cytochrome P_{450} enzyme system to a highly reactive intermediate (Ki-Tea *et al*., 2005).

It has been suggested that this toxic intermediate is the trichloromethyl radical ($CCl_3^{\bullet}$) producing maximum damage to liver (Recknagel *et al*., 1991. The free radicals can react with sulfhyhryl groups such as glutathione (GSH) and protein thiols. The covalent binding of trichloromethyl free radical to cell protein is considered the initial step in chain events which eventually leads to membrane lipid peroxidation and finally necrosis (Brattin *et al*., 1985; Recknagel *et al*., 1989, 1991).

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The association between diet rich in vegetables and decreased risk of cardiovascular disease and certain forms of cancer is supported by considerable epidemiological evidence (Lampe, 1999; Block *et al*., 1992). Different studies have shown that free radicals present in human organisms cause oxidative damage to different molecules, such as lipids, proteins and nucleic acids and thus are involved in the initiation phase of some degenerative illnesses. As a consequence, those antioxidant compounds that are capable of neutralizing free radicals may play a vital role in disease prevention. The selected vegetables for this investigation have undergone preliminary phytochemical investigation and a variety of phytochemical antioxidants have been identified (Akindahunsi and Salawu, 2005 a, b).

However, scientific studies on the protective effects of these tropical vegetables against a number of hepatoxic agents are lacking. Therefore, the aim of this study was to evaluate the hepatoprotective effects of some selected vegetables commonly consumed in the south western part of Nigeria against carbon tetrachloride induced liver damage in albino rats, this would be expected to provide a useful information to validate the usefulness of these natural products as a good source of natural antioxidants

Materials and Methods

Materials

Collection and Preparation of Materials

Seven tropical vegetables (*Corchrous olitorous*, *Solanum macrocarpon*, *Occimum graticimum*, *Manihot utilisima*, *Telfairia occidentalis*, *Structium sparejanophora* and *Amaranthus caudatus*) were collected from a vegetable farm at Obafemi Awolowo University Ile-Ife, Nigeria. The edible portion was separated, cut into pieces, washed, dried and grinded into fine powder. One hundred gram of each powdered samples were successively extracted in pure ethanol which was evaporated using a rotary evaporator under reduced pressure (Table 1). The powdered extracts were stored in the refrigerator for further studies.

Animals

The animals were distributed evenly into 16 groups of 5 animals each into stainless steel cages under standard conditions (25°C, 60-70% relative humidity and 12 h photo period). All animals had free access to commercial diet and water throughout the period of study. Carbon tetrachloride (0.5 mL kg^{-1} body weight) and extracts were administered to the animals orally by gastric intubation.

Carbon Tetrachloride-induced Liver Damage

The first group received vehicle mineral oil (pure corn oil) which served as the control group. Group 2 received single dose of CCl_4 in mineral oil (1:1 v/v). Groups 3-9 in addition to CCl_4 received 24 h latter, a daily dose of 250 mg kg^{-1} body weight of each extracts for seven days while groups 10-16 were treated as in groups 3-9 above, but instead were given 500 mg kg^{-1} body weight. Animals were sacrificed 24 h after the last injection. Blood was collected, allowed to clot and serum separated. The liver was dissected out and used for biochemical studies.

Table 1: Percentage yield of ethanolic extracts of some selected tropical vegetables

Vegetable	Local names	Yield (g/100 g)
Telfairia occidentalis	Ugu	19.65
Manihot utilisima	Odo ege	23.17
Structium sparejanophora	Ewuro odo	27.17
Amaranthus caudatus	Tete	18.03
Occimum graticimum	Efinrin	20.59
Solanum macrocarpon	Igbagaba	27.90
Corchorous olitorus	Ewedu	18.72

Biochemical Studies

The blood was obtained from all animals by making incision on the jugular vein. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and used for the estimation of various biochemical parameters namely ALT, AST (Bergmeyer *et al*., 1978) total protein content was measured by the method of Lowry *et al*. (1951). After collection of blood samples, the rats were sacrificed and their livers excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. Ten percent (w/v) of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation measured in terms of Malondialdehyde (MDA) using the method of Olusesi (2002). The rest of the homogenate was used for the estimation of ALT, AST and total protein.

Statistical Analysis

Data were expressed as mean±SD and were analyzed by One-Way ANOVA test using SPSS statistical programme.

Results and Discussion

Table 2 and 3 shows the effect of the selected vegetable extracts on some biochemical indices; alanine transferase (ALT), aspartate transaminase (AST) and total protein level in the serum of albino rats fed with ethanolic extracts of some tropical vegetables at concentrations of 250 and 500 mg kg^{-1} body weight (b.w). Results presented on Table 2 and 3 show a remarkable increase in ALT (IU L^{-1}), AST (IU L^{-1}) and total protein (g L^{-1}) level in the carbon tetrachloride-treated animals. CCl_4-induced hepatic injuries are commonly used models for the hepatoprotective screening of plant materials (Slater, 1965; Plaa and Hewitt, 1972). CCl_4 has the ability of being converted to reactive metabolites N-actetyl-p-bezoquinoneimine (NAPQI) and Halogenated Free Radicals (HFR) by hepatic cytochrome P_{450} (Packer *et al*., 1978; Van de Straat *et al*., 1987).

Table 2: Effect of some selected vegetable extracts (250 mg kg^{-1} bw) serum ALT, AST and total protein in CCl_4 intoxicated rats

Group	Treatment	ALT(IU L^{-1})	AST (IU L^{-1})	Total protein (g L^{-1})
1	Control+CO	13.12±1.20	27.21±2.10	70.00±1.90
2	CO+CCl_4	40.60±3.50	80.60±5.10	73.20±1.87
3	CCl_4+CO+TOC	14.00±1.33	29.00±2.70	62.10±2.40
4	CCl_4+CO+MU	12.00±0.90	36.10±3.00	66.00±2.00
5	CCl_4+CO+SS	13.12±1.02	48.00±2.10	65.00±1.30
6	CCl_4+CO+OG	14.00±1.70	36.10±1.50	63.00±2.00
7	CCl_4+CO+SM	11.21±1.90	36.00±2.50	65.00±1.50
8	CCl_4+CO+COL	16.22±1.00	34.11±3.00	64.00±2.00
9	CCl_4+CO+AC	14.00±1.40	33.00±3.30	70.13±3.00

Means±standard deviation; n = 5

Table 3: Effect of some selected vegetable extracts (500 mg kg^{-1} bw) serum ALT, AST and total protein in CCl_4 intoxicated rats

Group	Treatment	ALT (IU L^{-1})	AST (IU L^{-1})	Total protein (g L^{-1})
1	Control+CO	13.12±1.20	27.21±2.10	70.00±1.90
2	CO+CCl_4	40.60±3.50	80.60±5.10	73.20±1.87
10	CCl_4+CO+TOC	18.00±1.06	52.00±3.50	71.00±3.10
11	CCl_4+CO+MU	14.00±1.00	41.00±1.50	70.00±2.80
12	CCl_4+CO+SS	16.00±1.50	59.00±2.20	69.00±2.30
13	CCl_4+CO+OG	17.00±1.30	44.00±2.30	68.00±2.40
14	CCl_4+CO+SM	13.00±1.20	52.00±2.60	72.00±2.10
15	CCl_4+CO+COL	21.00±1.30	47.00±1.50	73.00±1.90
16	CCl_4+CO+AC	19.00±1.00	40.00±2.50	74.00±3.10

Means±standard deviation; n = 5, CO = Corn oil, OG = *Occimum graticimum*, CCl_4 = Carbon tetrachloride, SM = *Solanum marcrocarpon*, TOC = *Telfairia occidentalis*, COL = *Corchorous olitorus*, MU = *Manihot utilisima*, AC = *Amaranthus caudatus*, SS = *Structium sparejanophora*

The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol) ensuring wide-spread propagation of the alkylation as well as peroxidation, causing damage to macromolecules in vital biomembranes (Pesh-Imam and Recknagel, 1977; Aldridge, 1981). The enhanced activities of the serum marker enzymes observed in CCl_4-treated rats; 40.60±3.50 (ALT), 80.60±5.10 (AST), 73.20±1.87 (total protein) and in liver homogenate marker enzymes; 1300.20±7.38 (ALT), 1600.00±13.69 (AST), 250.00±7.51 (total protein) compared to the control correspond to extensive liver damage induced by the toxin (Venukumar and Latha, 2002). The rise in serum and liver ALT, AST and total protein has been attributed to the damaged structural integrity of the liver (Pesh-Iman and Recknagel, 1977; Gilani and Jambaz, 1995). The serum maker enzymes as presented on Table 2 and 3 range from 11.21±1.90- 40.60±3.50 (ALT), 27.21±2.10- 80.60±5.10 (AST), 62.10±2.40-73.20±1.87 (total protein) and 13.12±1.20-40.60±3.50 (ALT), 27.21±2.10-80.60±5.10 (AST), 68.00±2.40-74.00±3.10 (total protein) at a dose of 250 and 500 mg kg^{-1} bw respectively. The decreasing values of ALT, AST and total protein in respect to the control are associated with the effect of the vegetable extract as free radical scavengers and the ability of the extract to reduce the concentration of trichloromethyl radical that produces the liver damage (Recknagel *et al*., 1989; Koop, 1992). At concentrations of 250 mg kg^{-1} bw, *Solanum macrocarpon* brings about the least reduction of ALT level after CCl_4 intoxication; the least reduction of AST and total protein are associated with *Telfairia occidentalis*. At a higher dose of 500 mg kg^{-1} bw, it was observed that the least reduction of ALT is associated with *Solanum macrocarpon*, *Amaranthus caudatus* brings about the least reduction in AST level while *Occimum graticimum* brings about the least reduction of the serum total protein. On the over all, all the vegetables resulted in a decreased biochemical indices evaluated with respect to the CCl_4 intoxicated rats. These tendencies are clear manifestation of the hepatoprotective effects of the studied vegetables.

The activities of aspartate aminotransferase, alanine amino transferase and total protein in the serum have been found to be a useful indicator of liver damage in the diagnosis and study of acute hepatic disease (He and Aoyama, 2003). Administration of extracts of *Manihot utilisima*, *Sructium sparejanophora*, *Solanum macrocarpon* at a dose of 250 mg kg^{-1} b.w were able to remarkably prevent the elevation of serum ALT while at a dose of 500 mg kg^{-1} bw only *Solanum macrocarpon* was able to prevent the elevation of serum ALT. *Telfairia occidentalis*, *Manihot utilisima*, *Structium sparejanophora*, *Occimum graticimum*, *Solanum macrocarpon*, *Corchorous olitorous* and*Amaranthus caudatus* were able to prevent the elevation of serum total protein at a dose of 250 mg kg^{-1} bw while at a dose of 500 mg kg^{-1} bw, *Manihot utilisima*, *Structium sparejanophora* and *Occimum graticimum* were able to prevent the elevation of the serum total protein. Inability of some vegetables to prevent the elevation of the serum enzyme makers even at higher doses might be due to the interfering effect of some bioactive components of the vegetables.

The activities of the liver enzyme makers and proteins are not located only in the serum but also in the liver (He and Aoyama, 2003). Table 4 and 5 therefore show the effects of the vegetable extracts at a dose of 250 and 500 mg kg^{-1} on liver homogenate biochemical markers (ALT, AST, total protein). Similar results were obtained for the liver enzyme makers and total protein as obtained in the serum where all the vegetable samples resulted in a reduced level of ALT, AST and total protein at a dose of 250 and 500 mg kg^{-1} bw. However, the reduction of the enzyme makers was at different rates. Table 4 revealed that *Corchorous olitorous* was the only extract that could prevent the elevation of ALT, *occimum graticimum* was able to prevent the elevation of AST, while none of the vegetables was able to prevent completely the elevation of total protein at a dose of 250 and 500 mg kg^{-1} bw. Results in Table 5 show that *Corchorous olitorus* is also able to prevent completely the elevation of ALT.

The observed curative effects against CCl_4 induced hepatotoxicity may be attributed to the reported presence of some antioxidant phytochemicals and the evaluated antioxidant indices of these tropical vegetables (Akindahunsi and Salawu, 2005a, b).

Table 4: Effect of some selected vegetable extracts (250 mg kg^{-1} bw) liver homogenate ALT, AST and total protein in CCl_4 intoxicated rats

Group	Treatment	ALT (IU L^{-1})	AST (IU L^{-1})	Total protein (g L^{-1})
1	Control+CO	900.00±3.16	1270.00±10.05	150.00±2.70
2	CO+CCl_4	1300.00±7.38	1660.00±13.69	250.00±7.51
3	CCl_4+CO+TOC	940.00±3.26	1150.00±5.57	175.00±4.30
4	CCl_4+CO+MU	960.00±5.03	1270.00±9.38	180.00±2.00
5	CCl_4+CO+SS	1020.00±4.25	1440.00±5.51	150.00±3.12
6	CCl_4+CO+OG	980.±4.40	1270.00±4.95	185.00±3.00
7	CCl_4+CO+SM	960.00±2.29	1530.00±4.99	185.00±4.15
8	CCl_4+CO+COL	900.00±3.05	1400.00±6.95	170.00±3.85
9	CCl_4+CO+AC	954.00±3.72	1150.00±9.50	165.00±3.39

Means±standard deviation; n=5

Table 5: Effect of some selected vegetable extracts (500 mg kg^{-1} bw) liver homogenate ALT, AST and total protein in CCl_4 intoxicated rats

Group	Treatment	ALT	AST	Total protein
1	Control+CO	900.00±3.16	1270.00±10.05	150.00±2.70
2	CO+CCl_4	1300.00±7.38	1660.00±13.39	250.00±7.51
10	CCl_4+CO+TOC	1020.00±7.50	1530.00±8.50	190.00±6.70
11	CCl_4+CO+MU	1025.00±7.40	1500.00±12.03	195.00±3.58
12	CCl_4+CO+SS	1100.00±6.39	1660.00±8.69	165.00±5.50
13	CCl_4+CO+OG	1100.00±6.81	1670.00±7.99	210.00±4.41
14	CCl_4+CO+SM	1040.00±3.20	1660.00±5.76	190.00±6.82
15	CCl_4+CO+COL	900.00±3.05	1400.00±6.95	170.00±3.85
16	CCl_4+CO+AC	1030.00±8.80	1440.00±11.38	200.00±4.59

Means±standard deviation; n = 5

Table 6: Effect of some selected vegetable extracts on lipid peroxide content (nmol TBARS/mg Liver Protein)

Group	Treatment	MDA (250 mg kg^{-1} bw)	MDA (500 mg kg^{-1} bw)
1	Control+CO	45.00±0.07	45.00±0.07
2	CO+CCl_4	82.00±0.02	82.00±0.07
3 and 10	CCl_4+CO+TOC	46.00±0.08	52.00±0.06
4 and 11	CCl_4+CO+MU	44.00±0.03	47.00±0.07
5 and 12	CCl_4+CO+SS	50.00±0.01	53.00±0.09
6 and 13	CCl_4+CO+OG	45.00±0.07	60.00±0.10
7 and 14	CCl_4+CO+SM	52.00±0.06	52.00±0.06
8 and 15	CCl_4+CO+COL	49.00±0.08	56.00±0.06
9 and 16	CCl_4+CO+AC	47.00±0.03	51.00±0.08

Means±standard deviation; n = 5, CO = Corn oil, OG = *Occimum graticimum*, CCl_4 = Carbon tetrachloride, SM = *Solanum marcrocarpon*, TOC = *Telfairia occidentalis*, COL = *Corchorous olitorus*, MU = *Manihot utilisima*, AC = *Amaranthus caudatus*, SS = *Structium sparejanophora*

In order to further establish the antioxidative properties of these vegetables, *in vitro* lipid peroxidation experiment was carried out. The localization of radical formation resulting in lipid peroxidation was measured as Malondialdehyde (MDA) content in the liver homogenate as shown on Table 6. The MDA content in the liver homogenate increased in CCl_4 intoxicated rats compared to the control group (45.00±0.007-82.00±0.07). Although, similar to order biochemical indices evaluated, all vegetables bring about a reduction in the MDA value after administration of extract in addition to CCl_4. However, *Manihot utilisima* and *Occimum graticimum* were able to prevent peroxidation completely at a dose of 250 mg kg^{-1} bw while none was able to completely prevent peroxidation at a higher dose of 500 mg kg^{-1} bw. This might be due to the interference of the antiperoxidative principles by some other bioactive components of the vegetables.

The biochemical mechanism involved in the development of CCl_4 hepatoxicity has been long investigated. It is generally believed that the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4-induced liver lesion (Castrol *et al*., 1974; Mailing *et al*., 1974). This led to the investigation on the tropical vegetables which may have the potential to help in restoring liver functions and indirectly participate in detoxifying process as a natural source of antioxidant. Plants that exhibit Ca^{2+} channel blocking activity usually exhibit hepatoprotective activity against CCl_4 induced liver damage. By implication, the hepatoprotective activity of the selected vegetables against CCl_4 induced liver damage may be attributed to its Ca^{2+} channel blocking activity though direct evidence of this mechanism is lacking. The result of this study provides scientific support for the antioxidative potentials of the selected vegetables.

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