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Effects of Ethanolic Extract of *Gongronema latifolium* on Selected Tissues of Rabbits Exposed to Carbon Tetrachloride

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ABSTRACT: The present study was carried out to investigate the protective effects of ethanolic extract of the leaves of *Gongronema latifolium* on carbon tetrachloride-induced liver damage in rabbits. The experimental animals were divided into four groups with Group I being normal control animals and Group II Carbon tetrachloride-treated control animals. Animals in group III and IV were given ethanolic extract of *Gongronema latifolium* (200 mg/Kg. body weight and 400 mg/Kg body weight respectively for ten days prior to carbon tetrachloride challenge. One ml/kg of a 1:1 carbon tetrachloride: olive oil preparation was administered orally following an overnight fast to animals in groups II, III and IV on day 11 of the study. The results obtained revealed that CCl_4 caused a significant increase ($p < 0.05$) in the plasma levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, Lactate dehydrogenase and gamma glutamyl transferase. Conversely, the levels of these enzymes were significantly lower in the liver of CCl_4-treated animals relative to controls. Treatment with ethanolic extract of *G. Latifolium* provided significant degree of protection from the CCl_4-induced hepatotoxicity, with the 400mg/Kg body weight treatment being the more effective dose.

Keywords: *Gongronema latifolium*, Carbon tetrachloiride, Liver, ALT, AST, ALP

Introduction

Toxic chemical substances could cause serious health problems including damage to organs such as the liver(1). The liver is the most important organ as it plays a pivotal role in regulating various physiological processes in the body, especially in the detoxification of toxic substances. Carbon tetrachloride (CCl_4) has long been known as a model toxicant and has been the focus of many *in vitro* and *in vivo* toxicological studies (2).The massive destruction associated with carbon tetrachloride-induced tissue damage is due to the generation of free radicals (3). Carbon tet as it is commonly called, is metabolized by cytochrome P-450 in the endoplasmic reticulum to form trichloromethyl free radicals (2). The toxicity of this reactive compound formed, results from covalent interactions with critical target molecules such as lipids, proteins and the alteration of the target molecules through secondary bond formation.
Although a number of other organs such as the kidney, lungs, brain and testis may be affected by Carbon tetrachloride toxicity, the liver remains the major target organ due to its high content of cytochrome P-450 (4). Antioxidant property is claimed to be one of the mechanisms of hepatoprotective agents, especially those of plant origin (5).
Though the body has evolved a way of tackling these substances, the body defense system needs to be enhanced and activated (6). *Gongronema latifolium*, commonly known as Bush Buck, is a plant found predominantly in the South-East of Nigeria. It is commonly known as *'utazi'* and *'arokeke'* in the south-eastern and south-western parts of Nigeria respectively (7) where it is widely used in folk medicine and as a vegetable (8). *Gongronema latifolium* is a herbaceous shrub with yellow flowers and possesses a hollow stem which yields a characteristic milky exudate when cut. The plant has broad ovate leaves that are widely cordate at the base (9). The medicinal values of the plant are believed to be due to the presence of bioactive compounds such as alkaloids, tannins, flavonoids and glycosides. These compounds are thought to confer varied pharmacological effects on the plant (10).
Right from his first awakening, man has sought for ways of preventing and controlling diseases and pain with inspiration from his natural environment. Several plant materials by instincts were used to combat various disease conditions (11) even without the medicinal effects of this plant being considered. With advances in western scientific methods, most of these 'so-called' medicinally important plants came under chemical scrutiny and were tested for their ability to exhibit the professed healing effects both *in vivo* and *in vitro* before they could be certified to have protective effects (12).
The medicinal importance of *G. latifolium* cannot be over emphasized, as it plays a vital role in the treatment and prevention of a variety of health related problems including *Diabetes mellitus* (13); high blood pressure, loss of appetite, dysentery, stomach pains, worm infections, cough and malaria fever (14; 15). While it is postulated that extracts of *G. latifolium* could have hypoglycaemic, hypolipidaemic, antibacterial (16) and antioxidative effects (17); not many scientific studies have been done to establish these effects (18).
The primary concern of this study was to investigate the protective effects of the ethanolic extract of the leaves of *Gongronema latifolium* on carbon tetrachloride-induced hepatotoxicity as well as possible complications involving other organs such as the kidney and heart. Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in the plasma as well as in the tissues mentioned were used as markers of tissue damage.

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Methodology

Preparation of Plant Material
Fresh leaves of *Gongronema latifolium* were obtained from New Benin market, Benin City. The leaves were taken to the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State for identification and authentication. Thereafter, the leaves were hand-plucked, sundried and pulverized by means of an industrial grinder.

Preparation of Ethanolic Extract of *G. Latifolium*
Six hundred grams of the pulverized leaves was dissolved in 4500ml of 70% ethanol solution. The mixture was stirred repeatedly by means of a glass rod and kept on the laboratory bench for 24 hours before filtering. The filtrate was concentrated with the aid of a rotary evaporator and an oven set to 40^0C. The concentrated extract was then stored in a refrigerator at 4^0C. Stock solution of 200mg/ml was subsequently prepared by dissolving 20g of dried extract in 100ml of distilled water. This was used for the treatment of animals as required.

Experimental Animals
A total of twenty rabbits of the New Zealand White strain (average weight 1.5kg and aged between 5 -6 months) were used for the experiment. These were purchased from a local breeder in Benin City. The animals were allowed two week acclimatization to the animal house of the Department of Biochemistry, University of Benin before commencement of the experiment. They were kept in standard cages, and were fed *ad libitum* with water and standard pelletized growers feed (product of Bendel Feeds and Flour Mill, Ewu, Edo State, Nigeria) for the duration of the study.

Experimental Design and Animal Treatment
Experimental animals were divided into 4 groups of n=5. Group I which served as control was CCl_4 and extract free; group II was treated with only CCl_4 while groups III and IV received both CCl_4 and extract (200 $mgKg^{-1}$bw and 400 $mgKg^{-1}$bw respectively). Carbon tetrachloride (1$mlKg^{-1}$bw of a 1:1 CCl_4:olive oil) was administered orally by gavage on day 11 following 10 days of pre-treatment with ethanolic extract of *G. latifolium*

Preparation of Plasma Samples and Tissue Homogenate
All animals were sacrificed on day 12 following an overnight fast. Blood samples were collected in lithium heparin bottles, centrifuged for 5 minutes at 3,500rpm to obtained neat plasma samples. In addition, the animals were dissected and the liver, kidney and heart surgically removed and immediately blotted with Whatman filter paper to remove any trace of blood. Approximately 1gram portion of each organ was homogenized in 5mls of ice-cold normal saline. The homogenates were centrifuged for 10 minutes at 3500rpm. Then, using a Pasteur pipette, the supernatant was carefully separated from the pellet. The pellet was discarded while the supernatant was kept frozen until required for biochemical assays, all of which were carried out within 48hours.

Biochemical Assays
All reagents and chemicals used in this work were of analytical grade. All the biochemical assays were carried out with the aid of commercially available test kits products of Randox Laboratories, U.K. In all instances, the manufacturer's instructions were strictly adhered to.

Statistical Analysis
The data obtained were expressed as mean ± standard error of mean (SEM). The results were statistically analyzed using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Significant differences between the treatment means was set at $p < 0.05$.

Results

The results obtained from this study are presented in Tables 1 - 6. Treatment with CCl_4 resulted in significant increases ($p<0.05$) in plasma levels of ALT, AST, ALP, LDH and GGT when compared with controls. Also the levels of these enzymes were significantly lower ($p<0.05$) in the liver of CCl_4-treated animals relative to control. However, only the level of ALP was significantly reduced ($p<0.05$) in the kidney of carbon tetrachloride-treated animals when compared with controls. No significant changes ($p>0.05$)were observed in parameters investigated in the heart. Treatment with *G. latifolium* at both doses tested in this study offered significant degree of protection against liver damage. In addition, the 400mg/Kg body weight dose proved to be the more effective dose.

Table 1: The effect of *Gongronema latifolium* on carbon tetrachloride-induced biochemical changes in plasma enzyme activities

Group	Treatment	Plasma		
		ALT (U/L)	AST (U/L)	ALP (U/L)
I	Normal Control	23.40 ± 2.1^{a}	$2\ 6.73 \pm 1.06^{a}$	21.62 ± 1.08^{a}
II	CCl_4-treated control	52.70 ± 3.4^{b}	53.91 ± 2.42^{b}	46.40 ± 2.35^{b}
III	*G. latifolium* (200mg) + CCl_4	37.46 ± 1.26^{c}	35.07 ± 2.24^{c}	30.07 ± 1.08^{c}
III	*G. latifolium* (400mg) + CCl_4	28.00 ± 0.68^{a}	$26.80. \pm 1.14^{a}$	22.73 ± 0.94^{a}

Values are the arithmetic mean ±SEM; n = 5. Values on the same column with different superscripts differ significantly ($p<0.05$)

Table 2: The effect of *Gongronema latifolium* on carbon tetrachloride-induced biochemical changes in plasma LDH and GGT activities

Group	Treatment	Plasma	
		LDH (U/L)	GGT(U/L)
I	Normal Control	12.46 ± 1.14 [a]	9.45 ± 0.42 [a]
II	CCl_4-treated control	31.70 ±2.12 [b]	23.08 ±1.3 1 [b]
III	*G. latifolium* (200mg) + CCl_4	24.43 ± 1.24 [c]	16.07 ± 1.24 [c]
IV	*G. latifolium* (400mg) + CCl_4	19.56± 1.18 [c]	11.76. ±1.04 [a]

Values are the arithmetic mean ±SEM; n = 5. Values on the same column with different superscripts differ significantly (p<0.05)

Table 3: The effect of *Gongronema latifolium* on carbon tetrachloride-induced biochemical changes in Liver LDH and GGT activities.

Group	Treatment	Liver	
		LDH (U/g tissue)	GGT(U/g tissue)
I	Normal Control	2.15 ± 0.12 [a]	1.09 ± 0.04 [a]
II	CCl_4-treated control	1.12 ± 0.09 [b]	0.43 ± 0.02 [b]
III	*G. latifolium* (200mg) + CCl_4	1.46 ± 0.11 [b,c]	0.67 ± 0.02 [c]
IV	*G. latifolium* (400mg) + CCl_4	1.76. ± 0.10 [a]	0.86. ±0.03 [d]

Values are the arithmetic mean ±SEM; n = 5. Values on the same column with different superscripts differ significantly (p<0.05)

Table 4: The effect of *Gongronema latifolium* on carbon tetrachloride-induced biochemical changes in liver ALT, AST and ALP activities

Group	Treatment	Liver		
		ALT (U/g tissue)	AST (U/g tissue)	ALP (U/g tissue)
I	Normal Control	0.27 ± 0.07 [a]	0.42 ± 0.02 [a]	0.53 ± 0.021 [a]
II	CCl_4-treated control	0.13 ± 0.04 [a]	0.24 ± 0.02 [b]	0.27 ± 0.024 [b]
III	*G. latifolium* (200mg) + CCl_4	0.19 ± 0.09 [a]	0.30± 0.01 [b,c]	0.36 ± 0.013 [c]
III	*G. latifolium* (400mg) + CCl_4	0.25 ± 0.06 [a]	0.36 ± 0.004 [c,d]	0.47 ± 0.020 [a]

Values are the arithmetic mean ±SEM; n = 5. Values on the same column with different superscripts differ significantly (p<0.05)

Table 5: The effect of *Gongronema latifolium* on carbon tetrachloride-induced biochemical changes in kidney enzyme activities

Group	Treatment	Kidney		
		ALT(U/g tissue)	AST(U/g tissue)	ALP(U/g tissue)
I	Normal Control	0.281 ± 0.022 [a]	1.046 ± 0.055 [a]	0.354 ± 0.02 [a]
II	CCl_4-treated control	0.264 ± 0.086 [a]	0.948 ±0.027 [a]	0.262±0.01 [a]
III	*G. latifolium* (200mg) + CCl_4	0.296 ± 0.062 [a]	0.906± 0.053 [a]	0.294 ± 0.02 [a]
III	*G. latifolium* (400mg) + CCl_4	0.316 ± 0.04 7 [a]	1.152 ± 0.074 [b]	0.314 ± 0.02 [a]

Values are the arithmetic mean ±SEM; n = 5. Values on the same column with different superscripts differ significantly (p<0.05)

Table 6: The effect of *Gongronema latifolium* on carbon tetrachloride-induced biochemical changes in Heart ALT, AST and ALP activities

Group	Treatment	Heart enzymes		
		ALT (U/g tissue)	AST (U/g tissue)	ALP (U/g tissue)
I	Normal Control	0.165 ± 0.010 [a]	0.487± 0.015 [a]	0.213 ± 0.05 [a]
II	CCl_4-treated control	0.154 ± 0.010 [a]	0.497 ±0.019 [a]	0.218 ± 0.03[a]
III	*G. latifolium* (200mg) + CCl_4	0.149± 0.019 [a]	0.47± 0.016 [a]	0.198± 0.03 [a]
III	*G. latifolium* (400mg) + CCl_4	0.168 ± 0.015 [a]	0.484 ± 0.011 [a]	0.226 ± 0.04 [a]

Values are the arithmetic mean ±SEM; n = 5. Values on the same column with different superscripts differ significantly (p<0.05)

Discussion

In this study, liver damage was experimentally induced by the administration of a single oral dose of 1ml/kg body weight of a 1:1 carbon tetrachloride: olive oil preparation to animals pre-treated with ethanolic extract of *Gongronema latifolium* over a period of 10 days. The levels of the liver marker enzymes: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase were studied and compared with those of the normal control and carbon tetrachloride-treated animals. The results obtained are consistent with (19) who reported a significant increase in the plasma levels of these liver marker enzymes. Similarly, other workers (20) have also shown that the level of alanine aminotransferase was significantly increased ($p < 0.05$) in plasma after administration of carbon tetrachloride. However, in the latter report, the level of aspartate aminotransferase and alkaline phosphatase were not significantly altered ($p > 0.05$).

The data from the present study seem to suggest that hepatic involvement precedes renal involvement, and that the heart appears to be sufficiently shielded from CCl_4-induced insults on the organs studied. This is so because while marked changes were observed in levels of these enzymes in the liver, only ALP was significantly reduced in the kidney and none of the parameters investigated in the heart was significantly altered. This observation may be explained at least in part by the acute nature (24hours) of the exposure to CCl_4. Chronic exposures may alter this pattern and produce noticeable changes in both the kidney and heart of affected animals, probably as secondary events compounded by hepatic complications (2,4).

The massive destruction associated with carbon tetrachloride-induced tissue damage is due to the generation of free radicals (3). Carbon tetrachloride is metabolized in the endoplasmic reticulum to form trichloromethyl free radical. The toxicity of this reactive compound formed results from covalent interactions with critical target molecules such as lipids, proteins and from the alteration of the target molecules through secondary bond formation. Some plant extracts are able to protect against carbon tetrachloride-induced oxidative stress by lowering the levels of induced lipid peroxidation (21). The elevation in the plasma levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase are sensitive indicators of liver injury. The enzymes are good indicators of liver cytolysis and thus their elevated level in the plasma is an indication of organ dysfunction. Gao *et al.* (22) reported that an increased level of alanine aminotransferase in the plasma is an important index of cell membrane damage while aspartate aminotransferase indicates mitochondrial damage since over eighty percent of this enzyme is found in the mitochondria. Alkaline phosphatase is a marker enzyme for the plasma membrane and endoplasmic reticulum as it is located predominantly in the microvilli of the bile canaliculi. Hence, an increase in plasma alkaline phosphatase activity reflects pathological alterations in biliary flow.

Pre-treatment with the plant extract at 200 and 400 mg/kg body weight over a period of ten (10) days offered some degree of protection in a dose-dependent manner. It is likely that the protective effects of *G. latifolium* observed in this study are connected to its anti-oxidant properties. This is conceivable since CCl_4-induced hepatorenal damage is thought to progress via a free radical mediated mechanism (3). Agents with known anti-oxidant property have been shown to exhibit protective effects against CCl_4-induced damage. These include vitamin E (23) and vitamin C (24). Plants with antioxidant activity that have been tested against CCl_4-induced liver injury include *Phyllatus amarus* (19), *Ficus hisbida* (1), *Carica papaya* (25). Nwanjo *et al*, (17) reported the anti-lipid peroxidative effect of *G. latifolium* in streptozotocin diabetic rats. This is in agreement with the earlier report of antioxidant activity of the plant in non insulin-dependent *diabetes mellitus* (7). The pharmacological activity of *G. latifolium* may not be unconnected with the rich presence of bioactive phytochemicals such as alkaloids, Tannins, glycosides, flavonoids and polyphenols (26) Although this was not demonstrated in this study, it is also possible that *G. latifolium* possesses some ability to block or inhibit the bioactivation of carbon tetrachloride as was earlier reported for *Ficus hisbida* (1). While the precise mechanism of action of ethanolic extract of *G. latifolium* remains to be completely resolved, it is however not in doubt that the plant possesses significant potentials against CCl_4-induced hepatotoxicity.

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